Nutritive Value, Polyphenol Constituents and Prevention of Pathogenic Microorganism by Different Resin Extract of *Commiphora myrrh*

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Abstract

The resin extract of *Commiphora myrrh* is Widely used in the folk medicine. The studying myrrh resin extract include moisture. minerals such as (Ca, Fe, Mg, Na, Cu and Zn), protein, total fat and crude fiber. In this study used Muffle furnace, Kjeldahl methods Soxlet and atomic absorption. HPLC using to evaluating Polyphenol constituents of myrrh different resin extract (ethanol, ethyl acetate, petroleum ether and chloroform) as Conc. (µg / g) and in all extract (ethanol, ethyl acetate and petroleum ether and chloroform) it contained Chlorogenic acid, gallic acid Catechin, Coffeic acid, caffeine, Syringic acid, Coumaric acid, Ferulic acid, Naringenin, 4’.7-Dihydroxyisoflavone, Cinnamic, Propyl Gallate Vanillin, Quercetin and Acid Ellagic acid in different concentration percentage and area The effect of Commiphora myrrh (ethanol, ethyl acetate, petroleum ether and chloroform) resin extract against four different pathogenic bacteria *Salmonella typhimurium*, *Pseudomona aeruginosa*, *Escherichia coli*, and *Bacillus cereus*, were examine by Mueller Hinton Agar and measuring inhibition zone (diameter mm), show that there were significant different among bacteria and different method of extract. All different *Commiphora myrrh* seed extract (aqueous, ethyl acetate and petroleum ether) have high activity against Candida albicans fungus. The study was conducted to identified the *Commiphora myrrh* nutritive value, polyphenol Compound and the activity against bacteria and fungi.

Keywords: *Commiphora myrrh*, nutritive value, poly phenol constituent, antimicrobial, antifungal

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INTRODUCTION

Commiphora myrrh are small tree family Burseraceae. Myrrh native to the northeastern Africa, Somalia, Madagascar, India, Ethiopia, Iran, and Thailand1-7. Myrrh traditionally widely used in folk medicine, which has no toxicity8,9, myrrh resin was used in many medicinal purposes10, Myrrh used for inflammations of the mouth, throat is highly effective antiseptic astringent, has widely used in digestive tract diseases11-14. Its antibacterial14,15. Myrrh have antifungal activity16 anti-parasitic, detoxifying and reduced inflammation17,18. It can be used in remedy for chronic sinusitis. Myrrh is used in skin problems rashes, acne, and inflammatory also used in healing gastric ulcer or skin injury as powder, or essential oil19,20. It play an important role in cosmetics and perfumery.

MATERIAL

Commiphora myrrh purchase in the super market and identified in the Department of Biology, Science and Humanity College in Al-kharj, Prince Sattam bin Abdul-Aziz.

Microorganism

Microorganisms used in this work were obtained from laboratories of Microbiology “National Research Centre, Khartoum Sudan. The identification of bacterial by conventional biochemical methods21 according to the standard microbiology techniques. These microbes were, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus and Salmonella typhimurium.

METHODS

Moisture content

Oven used for determination of moisture content22.

Ash content

Muffle furnace used for determination of ash%23.

Determination of total fat

A Sox let extractor used for determination of fat23.

Determination of crude fiber

Determination according to the method described by Official methods of analysis of the association of analytical Chemists23.

Determination of protein as total nitrogen

The Kjeldahl method used for determination of protein23.

Determination of minerals

Atomic absorption spectrophotometry used for determination of the minerals23.

Determination of total carbohydrate

According to the method described by Official methods of analysis of the association of analytical Chemists23.

Preparation of plants extracts

100 g, of resin plant were weighed, and subjected to different extraction solvents separately extracted with ethanol 80% at 60°C for 2h in a Sox let apparatus ethyl acetate 80% at 50°C - 60°C for 2 h, petroleum ether 80% at 60°C for 2h Chloroform 80% at 50°C- 60°C for 2 h. The all solvents extract were evaporated by a Buchi Rotary evaporator under reduced pressure, also the resin plant was extracted by distilled water over night at room temperature (25-30°C) filtered and dried24.

HPLC conditions

The Agilent series 1260, Kromasil C18 column (4.6 mm x 250 mm i.d., 5 μm) were used for the separation in HPLC analysis. The mobile phase used consisted of water (A) and 0.1% trifluoro-acetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The column temperature was maintained at 35°C. The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μl for each of the sample solutions.

Mueller Hinton Agar

MHA (Mueller Hinton Agar) (Becton Dickinson M. D USA), media was prepared according to the manufacturer’s instruction. Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. Concentrations of 12.5, 25, 50 and 100mg/ml prepared from the seed different extract (aqueous, ethyl acetate and petroleum ether) agar were used for antibacterial analysis. Petri dishes prepared by agar and allow to solidify. Each plate was then seeded with a test bacterium. Four holes were made in each of the plate with a sterile 2.0 mm diameter cork borer. Each of the
four holes was filled with a given concentration of the extract mixed with plane sterile agar. The plates were then incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured using a meter rule and the mean value for each organism was recorded.

Preparation of fungal organism

The fungal is culture as fallow at temperature 25°C for 4 days put in Peptone water, sterile normal saline used for the harvested growth mat of fungal, washed and suspended stored in refrigerator till used.

Statistical analysis

It was done according to Duncan, Multiple Range Test.

RESULTS AND DISCUSSION

Table 1. show the approximate nutritive constituent of Commiphora myrrh resin contain Moisture % (0.10) , ash% (15.40), fiber% (0.0), protein% (9.97), carbohydrates% (56.01), fat% (17.96). Table 2. show the minerals content of the myrrh (ppm) such as Ca (20.80), Fe (139.540), Mg (359.203), Na (39.1), Cu (0.654) and Zn (0.6561), (26,28). Fig. 1 and Table 3. show the 17 Polyphenol constituents of Commiphora myrrh ethanol resin extract according to concentration and area, Querectin gave the highest concentration (54.31Conc. (µg / ml = µg / 20 mg) while Pyro catechol and Ellagic acid gave (0.00%) Table 4. and figure 2 show the Commiphora myrrh resin extracted by ethyl acetate contained 13 polyphenol the highest concentration is Querectin (211.14 Conc. (µg / ml = µg / 15 mg) while the lower one is Cinnamic acid (3.41 Conc. (µg / ml = µg / 15 mg).

Table 3. Commiphora myrrh resin extract

|                | Area (µg/ml = (µg / g) | Conc. (µg/g) |
|----------------|------------------------|-------------|
| Gallic acid    | 67.35                  | 4.34        | 216.84     |
| Chlorogenic acid| 28.36                 | 1.75        | 87.56      |
| Catechin       | 61.17                  | 10.15       | 507.51     |
| Caffeine       | 83.83                  | 2.43        | 121.71     |
| Coffeic acid   | 99.76                  | 3.29        | 164.33     |
| Syringic acid  | 101.70                 | 3.82        | 190.96     |
| Rutin          | 171.24                 | 25.79       | 1289.50    |
| Pyro catechol  | 0.00                   | 0.00        | 0.00       |
| Ellagic acid   | 0.00                   | 0.00        | 0.00       |
| Coumaric acid  | 41.05                  | 0.92        | 45.79      |
| Vanillinn      | 46.57                  | 1.63        | 81.74      |
| Ferulic acid   | 176.56                 | 3.76        | 457.28     |
| Naringenin     | 207.26                 | 8.42        | 420.76     |
| Propyl Gallate | 824.35                 | 20.03       | 1001.61    |
| 4’.7Dihydroxyiso Flavone | 600.45 | 15.10 | 754.82 |
| Querectin      | 674.27                 | 54.31       | 2715.62    |
| Cinnamic acid  | 461.17                 | 3.76        | 187.84     |

Table 4. Commiphora myrrh resin extract

|                | Area (µg/ml = (µg / g) | Conc. (µg/g) |
|----------------|------------------------|-------------|
| Gallic acid    | 273.98                 | 20.37       | 1357.68    |
| Chlorogenic acid| 198.63                 | 12.82       | 854.34     |
| Catechin       | 234.47                 | 37.76       | 2517.42    |
| Coffeic acid   | 193.22                 | 6.67        | 444.43     |
| Syringic acid  | 311.13                 | 11.37       | 758.04     |
| Rutin          | 1037.40                | 134.54      | 8969.27    |
| Ellagic acid   | 1066.67                | 67.05       | 4470.27    |
| Coumaric acid  | 278.07                 | 11.67       | 778.15     |
| Vanillinn      | 304.74                 | 7.15        | 476.75     |
| Ferulic acid   | 395.72                 | 13.04       | 869.28     |
| Naringenin     | 1797.77                | 100.81      | 6720.83    |
| Querectin      | 530.56                 | 211.14      | 14076.01   |
| Cinnamic acid  | 375.73                 | 3.41        | 227.29     |
resin extracted by petroleum ether contained 13 Polyphenol constituents, the highest one is Querectin (78.44 Conc. (µg / ml = µg / 15 mg) while the lower one is Gallic acid and Coffeic acid (0.00 Conc. (µg / ml = µg / 15 mg) Table 6. and figure 4 show the Commiphora myrrh resin extracted by Chloroform contained 13 Polyphenol constituents, the highest one is Querectin (182.45 Conc. (µg / ml = µg / 15 mg) while the lower one is Gallic acid (0.00 Conc. (µg / ml = µg / 15 mg) In all extract, show that the Querectin have high concentration, many studies reported the presence of phenolic compounds prevent body against oxidation, cancer and inflammation[28-30]. The antibacterial activity of the Commiphora myrrh by different method (ethanol, ethyl acetate, petroleum ether) seed extract against four different pathogenic organisms Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium and Bacillus cereus and one fungus Candida albicans (the lowest concentration of the Commiphora myrrh seed extract is (12.5 mg/ml) and the highest one is (100 mg/ml) there were differences effect among bacteria, Table 7 shows the inhibition zone (in mm) for different concentrations of Commiphora myrrh ethanol resin extract, the highest inhibition zone was detected against Bacillus cereus (13.75) and have the lowest one is Pseudomonas aeruginosa (12.25). Table 8 show that the Commiphora myrrh resin extracted by ethyl acetate, the high inhibited

### Table 5. Commiphora myrrh resin petroleum ether extract (Conc. 15 mg/ml)

| Area                  | Conc. (µg/ml = µg / 15 mg) | Conc. (µg/g) |
|-----------------------|-----------------------------|--------------|
| Gallic acid           | 0.00                        | 0.00         |
| Chlorogenic acid      | 6.89                        | 29.66        |
| Catechin              | 9.35                        | 100.40       |
| Coffeic acid          | 0.00                        | 0.00         |
| Syringic acid         | 32.22                       | 78.50        |
| Rutin                 | 38.84                       | 335.78       |
| Ellagic acid          | 58.12                       | 243.57       |
| Coumaric acid         | 22.16                       | 62.02        |
| Vanillin              | 82.42                       | 128.94       |
| Ferulic acid          | 86.74                       | 190.55       |
| Naringenin            | 90.78                       | 339.38       |
| Querectin             | 197.10                      | 5229.17      |
| Cinnamic acid         | 41.65                       | 25.20        |

### Table 6. Commiphora myrrh resin chloroform extract (Conc. 15 mg/ml)

| Area                  | Conc. (µg/ml = µg / 15 mg) | Conc. (µg/g) |
|-----------------------|-----------------------------|--------------|
| Gallic acid           | 0.00                        | 0.00         |
| Chlorogenic acid      | 14.62                       | 62.87        |
| Catechin              | 16.81                       | 180.51       |
| Coffeic acid          | 19.79                       | 45.51        |
| Syringic acid         | 34.57                       | 84.24        |
| Rutin                 | 49.20                       | 425.35       |
| Ellagic acid          | 237.37                      | 994.79       |
| Coumaric acid         | 56.46                       | 157.99       |
| Vanillin              | 258.43                      | 404.29       |
| Ferulic acid          | 210.19                      | 461.71       |
| Naringenin            | 55.69                       | 208.20       |
| Querectin             | 458.47                      | 12163.41     |
| Cinnamic acid         | 114.01                      | 68.97        |

### Table 7. Inhibition zone (in mm) for different concentrations of Commiphora myrrh ethanol resin extract

| Microorganism     | Concentration of the of Commiphora myrrh ethanol resin extract (µg/disc) | Mean Microorganism |
|-------------------|-------------------------------------------------------------------------|-------------------|
| Salmonella typhimurium | 12                 13              14               15        100 13.5 |
| Pseudomonas aeruginosa | 10                12              12               15        12.25 |
| Escherichia coli    | 12                13              14               15        13.5  |
| Bacillus cereus     | 10                14              15               16        13.75 |
| Candida albicans    | 10                13              14               15        13    |
| Mean Commiphora myrrh ethanol resin extract | 10.8           13            13.8             15.2 |
zone against *Escherichia coli* (15.25), while the lower one against *Bacillus cereus* (11.75). Table 9 show that the *Commiphora myrrh* resin extracted by petroleum ether, the high inhibited zone against *Escherichia coli* (12.75) and the lower inhibition zone against *Salmonella typhimurium* (10.75). Table 10 show the inhibition zone (in mm) for different concentrations of *Commiphora myrrh* chloroform extract the high inhibited zone against *Escherichia coli* (12.50) and the lower inhibition zone against *Salmonella typhimurium* (31,32,33,34). All different *Commiphora myrrh* resin extract (ethanol, ethyl acetate petroleum ether and chloroform) have the high activity against *Candida albicans* fungus these results agree with those who obtained that their in
Fig. 1. *Commiphora myrrh* resin ethanol extract 80% (20 mg/ml)

Fig. 2. *Commiphora myrrh* resin ethyl acetate extract (Conc. 15 mg/ml)

Fig. 3. *Commiphora myrrh* resin petroleum ether extract (Conc. 15 mg/ml)
The Commiphora myrrh resin chloroform extract (Conc. 15 mg/ml) have potent effect against bacteria and fungi.

CONCLUSION

The Commiphora myrrh contain polyphenol compound had potent antioxidant, antibacterial and antifungal activity in all different leaf extract.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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