Preliminary Phytochemical Screening, and Antimicrobial Activities of *Cynomorium coccineum* L. Grown in Libya

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ATBSTRACT

***Cynomorium coccineum* L. locally known "tarthuth" belongs to the family Cynomoriaceae, and is distributed mainly in the Mediterranean region, it is used to treat dysentery and haemorrhoids, for nasal and uterine bleeding, as a tonic, laxative and astringent. In this study phytochemical screening carried out using methanolic extract, and, antifungal and antibacterial activities of *C. coccineum* stem were tested against fungi (*Candida albicans, Cryptococcus neoformens, Aspergillus niger* and *Aspergillus flavus*) and bacteria (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*) by the agar well diffusion method and minimum inhibitory concentration (MIC). The result revealed the presence of valuable phytochemicals including flavonoids, terpenoids, saponins, tannins, cardiac glycosides and phenols. The methanolic extract showed inhibitory activity against fungi *C. albicans,* and *C. neoformens.* The zones of inhibition for these two species were between (10.6±1.2 mm and 17±0.8 mm) for all concentrations of the extract, with lowest MICs of 125 mg/ml and 250 mg/ml respectively. The methanolic extract showed inhibitory of growth against bacteria *S. aureus* and *P. aeruginosa.* The zones of inhibition ranged from (12 ± 0.8 mm to 25.3 ± 0.4 mm) for all concentrations of the extract, with lowest MICs of 125 mg/ml and 62.5 mg/ml respectively. The fungi *A. niger, A. flavus* and the bacteria *E. coli, K. pneumonia* were resistant to all concentrations. The result of this study showed that the plant's stem contained phytochemical constituents, and further supports its potential use as an antimicrobial agent.**

Keywords: *Cynomorium coccineum* L., Antifungal, Antibacterial, Libya.

INTRODUCTION

Cynomorium coccineum L. a perennial, angiosperm, blackish-red, leafless parasitic, the plant appears deep red when young and becomes blackish-purple when flowering, belongs to the family Cynomoriaceae (Jafri, 1977; Daoud, 1985). The species is distributed mainly in the Mediterranean region; it grows in dry rock or sandy soil (Lebling, 2003; Zucca *et al*., 2013). It is found in Libya, in Tobruk, Nalut, Ghade, Wadi Al-athal, Ghadames and Zwara. Moreover, it is found in the

desert areas of Tunisia, Algeria, Morocco, Egypt, Syria, Iraq and Iran (Jafri, 1977). The plant is known by different vernacular names depending on the country, the most common being "Maltese mushroom" (Goncalves *et al.*, 2015). In Libya and Arabic countries, the popular name is "tarthuth" (Zucca *et al*., 2013; Lebling, 2003). Both Arabs and the Europeans were familiar with this species from the early middle ages *C. coccineum* was known for a wide range of medicinal properties and thus used to treat dysentery, and bleeding during pregnancy and was used as a tonic, laxative, and astringent It was prescribed in Malta to treat high blood pressure and irregular menstrual periods (Lebling, 2003). The dried mature spike of *C. coccineum* has been used to treat colic and stomach ulcers. In addition, in North Africa, consumption of three cups of a decoction of the plant's aerial parts before meals is used to treat hemorrhoids and diarrhea (Cui *et al*., 2013). In the present study a focus has been placed on phytochemical constituent and evaluation of antimicrobial activities of *C. coccineum* stem grown in Libya.

MATERIALS AND METHODS

Plant collection and identification

Aerial parts of *C. coccineum* L. were collected from Zwara (200 Km west of Tripoli, Libya) in March 2016. The plant was identified and classified by Dr. Mohammed Abu-Hadra, a plant taxonomist, at the Department of Botany, Faculty of Sciences, University of Tripoli. The voucher sample (D681711) was deposited at the herbarium, Department of Botany, University of Tripoli.

Preparation of extract (hot extraction)

The plant was gently cleaned using a brush to remove any soil residue. The plant was cut into small slices and dried under shade at room temperature, and then ground into a powder state using the grinder. Extraction of the dried plant material was performed using a Soxhlet apparatus, a method described by Handa (2008); 300g of powdered plant material was placed into a thimble of Soxhlet, then 1600 ml of 97% methanol (70 °C) was added to the flask of Soxhlet, and the material was extracted for about 24 hrs. The filtrate was collected in a glass jar. The jar was left open and placed away from direct sunlight to dry the sample in order to yield a crude extract. The residual extract was preserved in a sterile glass bottle until it was used for phytochemical screening, antifungal and antibacterial testing.

Preliminary phytochemical screening

Preliminary phytochemical screening of the methanolic extract obtained from *C. coccineum* was performed to identify for the presence of various chemical constituents according to the procedure

described by Tiwari *et al.* (2011). Detection of the phytochemical was based on visual observation following either a colour change or the formation of a precipitate after the addition of specific reagents

Antifungal and antibacterial activities

Tested microorganisms

The following fungi and bacteria were used for the experiment. Fungi: *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 204092) were obtained from the Department of Microbiology, Faculty of Medicine (University of Tripoli); *Aspergillus niger* and *Aspergillus flavus* were provided by Dr. Najat Elghariany, Department of Plant Protection, Faculty of Agriculture (University of Tripoli). Bacteria: Gram-negative *Escherichia coli* (ATCC25922), *Klebsiella pneumonia* (ATCC13883), *Pseudomonas aeruginosa* (NCTC6749) and Gram-positive *Staphylococcus aureus* (ATCC29213). All the mentioned bacteria strians were obtained from the Department of Microbiology, Faculty of Medicine (University of Tripoli).

Preparation of inoculum

Inoculum suspensions were prepared from recent cultures of selected bacteria/yeast and the bacteria was sub-cultured on Nutrient Agar, while the yeast was sub-cultured on Sabouraud Dextrose Agar, 4 or 5 pure colonies were then selected with an inoculating needle, transferred to a tube of 0.9% sterile saline and vortexed. The turbidity was corrected by adding sterile saline until the 0.5 McFarland turbidity standards of 1-2x 10^8 CFU/ml for bacteria and 1-5 x10^6 CFU/ml for yeast were achieved. The filamentous fungi were grown on Sabouraud Dextrose Agar at 28°C for 7- 14 days. The growth was then scraped aseptically, crushed and macerated thoroughly in 0.9% sterile saline. The inoculum size was adjusted to 0.4-5x10^5 spores/ml by microscopic enumeration with a cell-counting hemocytometer (Chandrasekaran and Venkatesalu, 2004).

Agar well diffusion method

The antifungal and antibacterial activities of the stem extract were evaluated using the agar well diffusion method. Petri plates were prepared by adding Sabouraud Dextrose Agar (for fungi) and Mueller-Hinton Agar (for bacteria), which were allowed to solidify. The plates were dried, and then 100µl of standardized inoculum suspension was poured in and spread uniformly. The excess inoculum was drained, and the plates were allowed to dry for 5 min. Wells (6 mm diameter) were punched in the agar plate using a sterile cork borer and then inoculated with 50µl of the plant extract at different concentrations (100 mg/ml, 300 mg/ml and 500 mg /ml). Antifungal standard AmphotericinB (10μ / disc) and antibacterial standard Ciprofloxacin (10μ /disc) were used as a positive control, and 20%

DMSO was used as a negative control. The plates were incubated at 28°C for 48hrs (for yeast), 28°C for 3-5 days (for filamentous fungi) and 37°C for 24 hrs (for bacteria). The zone of inhibition was measured in millimetres (mm). Tests were performed in triplicate (Balouiri *et al*., 2016).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was found using the macro-dilution method. The test extract was dissolved in 20% DMSO to obtain a stock solution. Two-fold serial dilutions of the methanolic extract yielding concentrations of 500, 250, 125, 62.5, 31.25 and 15.6 mg/ml respectively were prepared in test tubes containing suitable growth media (1ml Sabouraud Dextrose Broth for fungi and Mueller-Hinton Broth for bacteria). Then, 0.1ml of bacterial suspension (10^8 CFU/ml), yeast suspension (10^6 CFU/ml) and filamentous fungi suspension (10^5 spores/ml) were transferred to each tube. A negative control test tube containing only the test extract, and a further test tube containing both broth and microorganism were taken as a positive control. The tubes were incubated at 28 °C for 48hrs (for yeast), 28 °Cfor 3-5 days (for filamentous fungi) and 37 °C for 24 hrs (for bacteria). The tubes were examined for visual turbidity. The MIC values were taken as the lowest concentration that inhibited the visible growth of the sample. All the tests were performed in triplicate (Chandrasekaran and Venkatesalu, 2004).

Statistical analysis

The experimental measurements were carried out in triplicate are expressed as means ± standard deviation. Data were analyzed by Student's t-test using the Statview® version 5.0.1 software package (SAS Institute Inc, Abacus Concept, *Inc*., Berkeley, CA, USA). A p value of < 0.05 was considered significant.

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Preliminary phytochemical screening

Table1: Result of phytochemical screening of methanolic extract of *Cynomorium coccineum* L.

Phytochemicals test	Methanolic extract
Test for alkaloids	-
Test for flavonoids	+
Test for terpenoids	+
Test for saponins	+
Test for tannins	+
Test for phenols	+
Test for anthraquinones	-
Test for coumarians	-
Test for cardiac glycosides	+

Key: (-) not detected (+) detected.

Fungi species	**Concentration of methanolic extract(mg/ml)**				
Amphotriciam B (10μ / disc)	**DMSO**	**100**	**300**	**500**	
Table 2: Antifungal activity (zone of inhibition, m m) of methanolic extract of *Cynomorium coccineum* L.					
Candida albicans		10.6± 1.2	14.3± 1.2	17± 0.8	15.3±0.47
Cryptococcus neoformans		12.5 ±1.2	14.6±0.9	15±1.2	18 ±1.2
Aspergillus niger		–	–	–	18±1.0
Aspergillus flavus		–	–	–	19.04±0.6

Mean zone of inhibition of three assays, ± Standard deviation, (-) means no growth inhibition zone. Amphotericin B as a positive control, dimethyl sulfoxide (DMSO) as a negative control.

Bacteria species	**Concentration of methanolic extract(mg/ml)**				
Ciprofloxacin (10μ / disc)	**DMSO**	**100**	**300**	**500**	
Table 3: Antibacterial activity (zone of inhibition, m m) of methanolic extract of *Cynomorium coccineum* L					
Staphylococcus aureus		12±0.8	13±1.6	15±0.8	23.6±0.9
Pseudomonas aeruginosa		14.3± 0 . 9	21.3± 1 . 8	25.3± 0 . 4	30.3± 0.47
Klebsiella pneumonia		-	-	-	33.3±0.9
Escherichia coli		-	-	-	31.6±1.2

Mean zone of inhibition of three assays, ± Standard deviation, (-) means no growth inhibition zone. Ciprofloxacin as a positive control, dimethyl sulfoxide (DMSO) as a negative control.

Table 4: Minimum inhibitory concentration (MIC) of methanolic extract of *Cynomorium coccineum* L.

Fungi species	Concentration of methanolic extract(mg/ml)						MIC
	500	250	125	62.5	31.25	15.6	
Candida albicans	-	-	-	+	+	+	125
Cryptococcus neoformans	-	-	+	+	+	+	250

against fungi

(+) Growth, (-) No growth.

Table 5: Minimum inhibitory concentration (MIC) of methanolic extract of *Cynomorium coccineum* L.

against bacteria

Bacteria species	Concentration of methanolic extract(mg/ml)						MIC
	500	250	125	62.5	31.25	15.6	
Staphylococcus aureus	-	-	-	+	+	+	125
Pseudomonas aeruginosa	-	-	-	-	+	+	250

(+) Growth, (-) No growth.

DISCUSSION

Usually, phytochemical screening is the first step in research focusing on the isolation and purification of natural compounds. This step may provide information about the potential use of the plant material for medical and pharmaceutical purposes (Oikeh *et al.,* 2013). In the present study, tannins, terpenoids, saponins, phenols, flavonoids and cardiac glycosides were detected; this result

was in agreement with previous work reported by Al-Hamaidia (2016) on the methanolic extract of the *C. coccineum* stem collected in Saudi Arabia. In the present study, alkaloids, anthraquinones and coumarins were absent in the methanol extract. The absence of alkaloids also correlated with previous work (Al-Hamaidia, 2016). The results for terpenoids, phenols, cardiac glycosides and flavonoids in this research were in agreement with the findings reported by Rached *et al*. (2010) and Zucca *et al*. (2013). However, the absence of coumarins was not in agreement with the findings reported by Rached *et al*. (2010). Tannins have astringent properties; the presence of tannins suggests a plant's ability to be an anti-diarrheal and anti-haemorrhoidal agent (Pacome *et al*., 2014). This may be why the plant has been used as an astringent to treat haemorrhoids and diarrhoea (Cui *et al*., 2013). The presence of saponins in *C. coccineum* justifies the use of this plant to stop bleeding (Lebling, 2003), because saponins have the property of precipitating and coagulating red blood cells. Other properties of saponins include formation in aqueous solution, haemolytic activity and cholesterol binding properties (Gogoi and Islam, 2012). Flavonoids are potent water soluble, super antioxidant and free radical scavengers that prevent oxidative damage; they have anti-cancer and anti-inflammatory properties (Udu-Ibiam *et al*., 2014). Perhaps this explains why *C. coccineum* is used to treat ulcers in herbal medicine.

Antimicrobial activity is typically investigated to complement phytochemical study; a variety of laboratory methods can be used to evaluate the *in vitro* antimicrobial activity of plant extracts. The best known and most basic methods are the wells, disk diffusion, and broth or agar dilution methods (Balouiri *et al*., 2016). In the present study, antifungal and antibacterial activities of the *C. coccineum* methanol extract were investigated using the agar well diffusion and macro-dilution methods. The results are summarized in Tables 2,3,4 and 5.

For antifungal activity, the methanolic extract of *C. coccineum* was tested against *C. albicans, C. neoformans, A. niger* and *A. flavus*. The results showed that the extract had an inhibiting effect on the growth of *C. albicans* and *C. neoformans* (the range of the mean zone of inhibition was between 10.6± 1.2− 17±0.8 mm) that no a significant difference to positive control; Amphotericin B ($p<0.05$), whereas *A. niger* and *A. flavus* were totally resistant to all concentrations (100, 300 and 500 mg/ml) of the plant extract. Moreover, the results indicated that DMSO had no effect on the fungi as there was no zone of inhibition. Hence, an experiment conducted subsequently to determine the MIC against the most susceptible fungal strains (*C. albicans* and *C. neoformans*), the methanol extract showed MIC value of 125mg/ml against *C. albicans* and MIC value of 250mg/ml against *C. neoformans*. A similar investigation was carried out by Goncalves *et al*. (2015) on the antifungal activity of *C. coccineum* grown in Sardinia against, *C. albicans* and *C. neoformans* using broth dilution assays. They also found

evidence of *C. coccineum* activity against *C. albicans* and *C. neoformans,* in line with the result of the present study.

For antibacterial activity, the extract was tested against one strain of Gram-positive bacteria (*S. aureus*) and three strains of Gram-negative bacteria (*P. aeruginosa*, *K. pneumoniae* and *E. coli*). The results showed that the methanolic extract had an inhibiting effect on the growth of *S. aureus* and *P. aeruginosa*, (the range of the mean zone of inhibition was between $12\pm0.8-25.3\pm0.4$ mm). At a concentration of 500mg/ml, the extract had a zone of inhibition that no significant difference to positive control; Ciprofloxacin ($p<0.05$). Moreover, the concentrations of 100 and 300 mg/ml the methanolic extract showed a lower zone of inhibition against *S. aureus* and *P. aeruginosa* than the positive control; Ciprofloxacin ($p<0.05$). MIC values of 125mg/ml and 62.5mg/ml were found against *S. aureus* and *P. aeruginosa* respectively. In comparison, *E. coli* and *K. pneumoniae* were not susceptible to all the concentrations of the plant extract. In addition, the results indicated that DMSO had no effect on the bacteria as there was no zone of inhibition. In line with these findings, previous studies, have also reported growth inhibition of *S. aureus* by the extract of *C. coccineum* (Almussawi, 2014; Muhaisen *et al.,* 2016; Zucca *et al.,* 2016). The most sensitive bacterium of those tested was *P. aeruginosa*. In this case, the results of the present study is disagree with the findings reported by (Muhaisen *et al.* (2016) and Zucca *et al.* (2016). This discrepancy may be attributed to differences in the solvents used for extraction and in the methods followed for testing antibacterial activity; the geographical location the plant samples were collected from may also be a factor (Lulekal *et al.,* 2014). The aforementioned researchers (Zucca *et al.,* 2016; Muhaisen *et al.,* 2016) also showed that *E. coli* and *K. pneumonia* were not susceptible to extract of *C. coccineum*, a finding that matches the result of the present study but disagrees with the result reported by Almussawi (2014).

CONCLUSION

The phytochemical screening of the methanolic extract of the *C. coccineum* stem revealed the presence of flavonoids, terpenoids, saponins, tannins, phenols and cardiac glycosides. In addition, the methanolic extract of *C. coccineum* possesses growth inhibitory activity against *C. albicans, C. neoformans, S. aureus* and *P. aeruginosa*. These results could explain why this plant has been used in traditional medicine for the treatment of infections.

REFEARENCES

Al-Humaidi, J., 2016. Phytochemical screening, total phenolic and antioxidant activity of crude and fractionated extracts of *Cynomorium coccineum* growing in Saudi Arabia. *European Journal of Medicinal Plants*, *11*(4), 1-9.

Al-Mussawi, Ab., 2014. Screening for antibacterial activity of twenty-two Iraqi wild plants. *Global Advanced Research Journal of Microbial, 3* (9), 177-181.

Balouiri, M. N., Sadiki, M. and Ibnsouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6* (2), 71-79.

Chandrasekaran, M. and Venkatesalu, V., 2004. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *Journal of Ethnopharmacology, 91*(1), 105-108.

Cui, Z., Guo, Z., Miao, J., Wang, Z. Li. Q., Chai, X. and Li, M., 2013. The genus *Cynomorium* in China: An ethnopharmacological and phytochemical review. *Journal Ethnopharmacol, 147* (1), 1-15.

Daoud, H.S., 1985. Flora of Kuwait: Dicotyledonae, 2ed edition. London: *Kegan Paul International Ltd,* 63.

Gogoi, P. and Islam, M., 2012. Phytochemical screening of *Solanum nigrum* L. and S. *myriacanthus* Dunal from districts of upper assam, India. *International Organization of Scientific Research, Journal of Pharmacy, 2*(3), 455-459.

Gonçalves, M.J., Piras, A., Porcedda, S., Marongiu, B., Falconieri, D., Cavaleiro, C., Rescigno, A., Rosa, A. and Salgueiro, L., 2015. Antifungal activity of extracts from *Cynomorium coccineum* growing wild in Sardinia Island (Italy). *Natural Product Research, 29* (23), 2247-2250.

Handa, S.S., Khanuja, S.P.S., Longo, G., Rakesh, D.D., 2008. An overview of Extraction Techniques for Medicinal and Aromatic Plants. In: Handa,S.S. ed. Extraction Technologies for Medicinal and Aromatic Plants. Trieste: *United Nations Industrial Development Organization and International Center for Scinece and High Technology*, 23-24.

Jafri, S.M.H., 1977. Flora of Libya: Cynomoriaceae, Vol 17. Tripoli: *University of Al-Fatah press*, 1.

Lebling, R.W., 2003. The treasure of tarthuth. *Journal Saudi Aramco World, 54*(2), 12-17.

Lulekal, E., Rondevaldova, J., Bernaskova, E., Cepkova, J., Asfaw, Z., Kelbessa, E., Kokoska, L. and Van Damme, P., 2014. Antimicrobial activity of traditional medicinal plants from Ankober District, North Shewa zone, Amhara region, Ethiopia. *Pharmaceutical biology*, *52*(5), 614-620.

Muhaisen, H.M., Ab-Mous, M. M., Adeeb, F.M., Rtemi, A. A., Taba, O.M. and Parveen, M., 2016. Antimicrobial agents from selected medicinal plants in Libya. *Chinese Journal of Integrative Medicine, 22*(3), 177-184.

Oikeh, E.I., Oriakhi, K. and Omoregie, E.S., 2013. Proximate analysis and phytochemical screening of *Citrus sinensis* fruit wastes. *The Bioscientist, 1* (2), 164-170.

Pacome, O.A., Bernard,D.N., Sekou, D., Toseph,D.A., David, N.J., Mongomake, K. and Hilaire, K.T., 2014. Phytochemical and antioxidant activity of Roselle *(Hibiscus sabdariffa* L.) petal extracts. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5*(20), 1453-1465.

Rached, W., Benamar, H., Bennaceur, M. and Marouf, A., 2010. Screening of the antioxidant potential of some Algerian indigenous plants. *Journal Biological Sciences, 10* (4), 316–324.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H., 2011. Phytochemical screening and extraction: A review. *International Pharmaceutica Sciencia, 1*(1), 98-106.

Udu-Ibiam,O. E., Ogbu, O., Ibiam, U.A., Nnachi, A.U., Agah, M.V., Ukaegbu, C. O.,Chukwu, O.S., Agumah, N. B., Ogbu, K. I., 2014. Phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria. *International Organization of Scientific Research, Journal of Pharmacy and Biological Sciences, 9*(3), 86-91.

Zucca, P., Argiolas, A., Nieddu, M., Pintus, M., Rosa, M., Sanna, F., Sollai, F., Steri, D. and Rescigno, A., 2016. Biological activities and nutraceutical potentials of water extracts from different parts of *Cynomorium coccineum* L. "Maltese mushroom". *Journal of Food and Nutrition Sciences, 66* (3), 179.

Zucca, P., Rosa, A., Tuberoso, C.I., Piras, A., Rinaldi, A.C., Sanjust, E., Dessi, M.A. and Rescigno, A., 2013. Evaluation of antioxidant potential of "maltes mushroom" *Cynomorium coccineum* L. by means of multiple chemical and biological assays. *Nutrients, 5*(1), 149-161.