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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *Codiaeum variegatum* LEAVES

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ABSTRACT: Natural substances of botanical origin have been important in African traditional medical practice. They have been used for various illnesses such as infections. Infectious diseases caused by pathogenic bacteria affect many communities and the treatment was made difficult partly because of antibiotic resistant strains. Phytochemicals isolated from medicinal plants are known to be effective in treating bacterial infections. The antibacterial activities of the ethanol and water leaf extracts of *Codiaeum variegatum* were tested. Antibacterial effects of crude extracts were performed using modified Kirby-Bauer disc diffusion technique to determine the zone of inhibition. The extracts were tested for the antibacterial activities against Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Serratia marcescens).* The results demonstrated that both of ethanol and water leaf crude extracts of *Codiaeum variegatum* have shown strong zone of inhibition against *Serratia marcescens* (20 mm) and *Bacillus subtilis* (12 mm) compared to control. This medicinal plant could be developed into affordable and safe standardized herbal products and may serve as a source of new molecules for broad-spectrum antimicrobial agents.

Key words: Anti oxidant, water and ethanolic extract, antibacterial, total phenolic, total flavonoid.

INTRODUCTION

The World Health Organization reported that infectious diseases are responsible for one third of global mortality and that they still pose a major threat to public health in numerous countries (**WHO, 2017**). Their impact is more evident in developing countries due to the limited accessibility to medicines and the emergence of widespread drug resistance (**Macedo *et al.*, 2015**). The increasing resistance of microorganisms to available antimicrobial agents is one of the major concerns for scientists and clinicians worldwide, and has become a global problem in the last years (**Abad *et al.*, 2012**). Over the past decade, interest in the search for antimicrobial natural products has risen (**Ahmad *et al.*, 2006**). From all possible sources of natural products, plants are considered as the most promising product (**Macedo *et al.*, 2015**). Croton is the second most extensive genus of Euphorbiaceae with approximately 1250 species (**Costa *et al.*, 2012**). Currently, these species are known by presenting insecticidal, antimalarial, larvicidal (**Dória *et al.*, 2010**), antimicrobial, antiulcerogenic (**Almeida *et al.*, 2013**), molluscicidal, anthelminthic (**Camurça *et al.*, 2007**), and anti-inflammatory (**Nardi *et al.*, 2003**) activities.

Croton species are commonly used for treating diabetes, digestive disturbances, hypercholesterolemia, intestinal worms, fever, malaria and pain (**Dória *et al.*, 2010**). Moreover, vasorelaxant effects have been attributed to some species of the Croton genus, which have been reported to contain alkaloids, terpenoids (diterpenes, pentacyclic triterpenoids and steroids), proanthocyanidins, flavonoids, and other phenolic compounds (**Dória *et al.*, 2010**).

The aim of this work was to determine the antioxidant activity, total flavonoid, total phenolic, total alkaloid and anti bacterial activity of *Codiaeum variegatum* leaves.

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MATERIALS AND METHODS

Plant Material

The leaves of *Codiaeum variegatum* were collected from the farm of Faculty of Agriculture, Zagazig University. The plant was identified by botanical members of the Department of Botany, Faculty of Agriculture, Zagazig University, Egypt. The plant part were allowed to dry in a shady and well- aired place for 15 days, aerial parts of *Codiaeum variegatum* L., ware cut into small pieces, ground to fine powder using blender and kept for further investigations.

Chemicals

All solvents used throughout the present work were obtained from different companies. ABts, DPPH and Substrates were purchased from Sigma Chemical Co., St. Louis, USA.

Methods

Preparation of ethanolic extracts

The leaves of *Codiaeum variegatum* were extracted according to the method of **Panovska *et al.* (2005)** 50 g of dry powder were extracted with 500 ml of 70% ethanol in a screw-capped flask and shaken at room temperature for 24 hr. The extract was centrifuged at 5500 r.p.m for 10 min while the residue was re-extracted under the same conditions twice and filtered through filter paper. The 70% ethanol extract were concentrated under reduced pressure, lyophilized to obtain powder, and stored at -20°C until using for further study.

Preparing aqueous extract

About 500g of the ground leaves of Croton were soaked in distilled water for seventy two hr., and the residue was separated. Finally the aqueous extract was lyophilized and the final yield was 50 g. The resideu was kept in a clean vial and cooled in a refrigerator until use (**Nwangwa and Ekhoye, 2013**).

Chemical composition of plant sample

Determination of moisture, ash, crude fiber, crude protein and crude lipid were determined according to the method described in **AOAC (2005)**.

Total phenolic determination

Total phenolic compounds were determined according to the method described by **Ghasemzadeh *et al.* (2010).**

Determination of total flavonoids

The total flavonoid compounds were determined according to the method described by **Ahn *et al.* (2007)**.

Determination of total alkaloids

The total alkaloids compounds were determined according to the method described by **Adham (2015).**

Antioxidant Activity of *Codiaeum variegatum*

Free radical scavenging activity (RSA) DPPH assay

The RSA of the ethanolic extract of *Codiaeum variegatum* leaves was assayed with 1, 1-diphenyl-2picrylhydrazyl (DPPH) radicals dissolved in ethanol. RSA and the presence of hydrogen donors in ethanolic extract was examined by reduction of DPPH in ethanol. An ethanolic solution of DPPH radicals was freshly prepared. The radical, in the absence of antioxidant compounds were stable for more than 2 hr., of normal kinetic assay. For evaluation, 100 µl [80(µg/ml), 120(µg/ml), 150 (µg/ml), 200(µg/ml)] ethanolic extract of studied plant was mixed with 5 ml of ethanol solution of DPPH radicals and the mixtures were vortexed for 20 sec. at ambient temperature. Against a blank of pure ethanol without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells, using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of ethanol DPPH solution with or without sample (control) and the inhibition percentage was calculate according to **Lee *et al.* (2002)**, from the following equation:

Inhibition (%) = [(absorbance of control – absorbance of test sample) / absorbance of control] × 100

In vitro antimicrobial activity of resideu

Antimicrobial activities of the tested samples were determined using a modified Kirby-Bauer disk diffusion method **(Bauer *et al.*, 1996).** Plates inoculated with Gram (+) bacteria as *Bacillus subtilis,* ; Gram (-) bacteria as *Serratia marcescens* at 35 – 37°C for 24 – 48 hours., and then the diameters of the inhibition zone were measured in millimetres **(Bauer *et al.*, 1996)**. Standard discs of tetracycline (antibacterial

agent) served as positive control for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water and Ethanol 70%) were used as a negative control.

RESULTS AND DISCUSSION

Proximate Composition of *Codiaeum variegatum* leaves

The analyses of *Codiaeum variegatum* leaves content are recorded in Table 1. Results shoed that ash, crude protein, crude fat, crude fiber, carbohydrate and moisture valued 14.50%, 15.56%, 4.68%, 20.70%, 43.71% and 14.60%, respectively. Obtained results showed that the plant contained a considerable high amounts of ash, protein, fat, fiber and carbohydrate. These rustls are in harmmony with those obtemined by **Babatunde *et al.* (2017)** who stated that *Codiaeum variegatum* leaves contained crude ash (12.58 %), crude fat (6.52%), crude fiber (19.52%) and carbohydrate (57.77%).

The Total of Active Components in *Codiaeum variegatum* Leaves

Rice-Evans *et al.* (1996) and **Mattei *et al.* (1998)** reported that phenolic compounds have at least one aromatic ring which can carry the hydroxyl groups which can known as reducing agents. The natural antioxidants such as phenolics and flavonoids compounds have wide spectrum pharmacological effects like antibacterial, anti-allergic, neuroprotective activities, anti-inflammatory and anticancer, also protect plants from the attack of pathogenic microbial. **(Mohd *et al.*, 2012)** reviewed that the Croton plant is a great source of bioactive compounds like flavonoid and phenolic. Therefore, the total flavonoid and phenolic in the Croton plant are very important to discover the effect of Croton extract. **Veni *et al.* (2015)** reported that the *Codiaeum variegatum* is an ornamental plant and also known for its medicinal properties. The medicinal properties of plants are due to the phytochemicals. These phytochemicals are the secondary metabolites which are produced in adiquate quantity under stressed conditions, which allows the plant caused tolerance against adverse environmental factors. Dietary intake of these phytochemicals may promote health benefits, protecting against chronic degenerative disorders, such as cancer, cardiovascular and neurodegenerative diseases. This investigation shoewd that the phenolic contents was (65 mgGAE/ g), flavonoid was 27 mg QE/g. and total alkaloid was 3.71 g/100 g) **Veni *et al.* (2015)** reported that the *Codiaeum variegatum* quantitative estimation revealed that alkaloid is in between the range of (4.66 - 10.2%), flavonoids (33.1-37.63%), saponins (11.36 - 13.76%), phenolics (35.43-39.76%), tannins (10.5-15.32%), terpenoids (27.56-30.3%), also they found that the plant is rich in flavonoids, phenols and terpenoids. The *Codiaeum variegatum* is known to have medicinal properties like anticancer and anti-inflammatory properties. This study revealed the presence of medicinal active constituents of *Codiaeum variegatum* like flavonoids, phenols and terpenoids which are rich and may be contributing in providing the medicinal properties to the plant. The inflammation is mainly caused by cyclooxygenase and lipooxygenase pathway. Phenolic compounds were shown to inhibit the cyclooxygenase and lipooxygenase pathway **(Ferrandiz *et al.*, 1990; Ferrandiz and Alcaraz, 1991; Laughton *et al.*, 1991)**. Flavonoids are found to inhibit Ornithine decarboxylase enzyme, it is rate-limiting enzyme in polyamine biosynthesis, which has been correlated with the rate of DNA synthesis and cell proliferation in several tissues, hence inhibiting cell proliferation **(Makita *et al.*, 1996; Tanaka *et al.*, 1997a; Tanaka *et al.*, 1997b)**. Flavonoids inhibit growth of microorganism by depolarizing membrane, inhibiting DNA, RNA and protein synthesis **(Dzoyem *et al.*, 2013).** Study of flavonoids, phenols and terpenoids from this plant may help to exploit more of the medicinal properties of *Codiaeum variegatum leaves* (Table 2).

Radical Scavenging Activity (RSA) of *Codiaeum variegatum* leaves Extract

Many studies in the last ten years interested in the theory of free radical disease causation, especially in certain forms of cancer and vascular diseases. Because of the developments in the free radical field have guided us to the consideration on dietary agents, the natural antioxidant (especially vitamins E, A, and C), in a possible prophylactic and the role of the disease process. A free radical is a chemical

Table 1. Percentage of the proximate compositions of *Codiaeum variegatum* leaves

Parameter	Croton
Moisture (%)	14.6% (fw)
Crude proteins (%)	15.56% (Dw)
Crude fats (%)	4.68% (Dw)
Crude fibers (%)	20.7% (Dw)
Carbohydrates (%)	43.71%(Dw)
Ash (%)	14.5%(Dw)

Fw = fresh weight Dw= dry weight

Table 2. The total phenolic, total flavonoid and total alkaloid contents of *Codiaeum variegatum* Leaves

Plants extract	Total phenolic content (mg GAE/g sample DW)	Total flavonoid content (mg quercetin/g sample DW)	Total Alkaloid (g/100 g)
Croton	65.551	27.924	3.017

species that has unpaired electrons **Pryor *et al.* (2006).** These electrons, which made free radicals are very reactive and take section in chemical reactions with other components in cell such as proteins, complex carbohydrates, nucleic acids and lipids in the body **(Kohen and Nyska, 2002).** In the biological systems, free radicals are referred to reactive oxygen species (ROS), as the most biologically significant free radicals. ROS produced in cells include hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), and superoxide anion (O_2) **Pryor *et al.* (2006).**

Table 3 show the antioxidant activity of *C. variegatum* leaves. All the samples analysed showed an increase in antioxidant activity with increasing concentrations, thus exhibiting a concentration dependent pattern of free radical scavenging ability. The extract of *C. variegatum* recorded a stronge antioxidant activity. Members of the Euphorbiaceae plant family possess strong antioxidant activities which are greatly associated with the presence of phenolic compounds (**Shahwar *et al*., 2010**).

For instance, analysis of the leaf extract of *C. variegatum* cv spiral and royal-like by HPLC-DAD showed that ellagic acid, a phenolic compound may be responsible for its antioxidant activity (**Saffoon *et al*., 2014)**.

Antimicrobial Activity

The intensive use of antibiotics is often followed by the presence of resistant strains of microorganisms. In view of the resistance of bacteria to drugs, the search for natural compounds having antibacterial activity is an urgent one in order to cope with the harmful effects of these microorganisms. For these reasons, accordingly, in this research two extracts *i.e.*by ethyl alcohol and water were tested against different microorganisms Gram-positive *Bacillus subtilis* (G^+) and Gram negative bacteria.; *Serratia marcescens* (G^-). Inhibition zones are recorded as shown in Tables 4, 5. Control was in the same conditions. It was observed that control (DMSO) did not produce any inhibition zones. It is showed that all extracts gave rise to concentration dependent inhibition zones in the two types of bacteria (positive and negative Gram).

The results of the present study showed that the leaves water extract of *Codiaeum variegatum* inhibited the growth of tested isolate strongly; this may be due to presence of the phytochemical groups as mentioned in Table 2.

Table 3. DPPH scavenging activity of *Codiaeum variegatum* leaves extract

Plant extract concentration	80 (µg/ml)	120 (µg/ml)	150 (µg/ml)	200 (µg/ml)
DPPH (%)	50.45%	52.86%	55.13%	56.47%

Table 4. Effect of ethanolic extract resideu on the diameter inhibition zone (mm) of microorganisms

Concentration	Inhibition zone diameter (mm/mg sample)	
	***St.marcescens* (G-)**	***B. subtilis* (G+)**
Control : DMSO	NI	NI
1- (120mg/ml)	19	12
2- (60mg/ml)	17	10
3- (30mg/ml)	8	13
4- (15mg/ml)	13	7

Table 5. Effect of water extract resideu on the diameter inhibition zone (mm) of microorganisms

Sample	Inhibition zone diameter (mm/mg sample)	
	***St.marcescens* (G-)**	***B.subtilis* (G+)**
Control : DMSO	NI	NI
1- (800 mg/ml)	20	9
2- (400 mg/ml)	19	8
3- (200 mg/ml)	19	8
4- (100 mg/ml)	10	7

According to the findings, *Serratia marcescens* was found to be more sensitive to the extract than *Bacillus subtilis*. This result are in the same line with **Aylate *et al.* (2017)** who stated that extracts from croton plant showed stronger antibacterial activity also stated that the extract has shown the lowest inhibition zone (9.25±0.54mm) against *E. coli* and the highest inhibition zone (21.63 ± 0.02) was seen against *S. aureus*. In addition, the extract was significantly better than the effect of tetracycline against *S. aureus*. Relatively higher number of organs with lesion and death of mice during the trial was registered in experimental groups, infected with *E coli* than *S. aureus* infected ones, both treated with the plant extract. These findings present scientific evidences for the use of this plant as a remedy against disease caused by *S. aureus* and *E. coli* pathogens.

Conclusion and Recommendations

The results and discussion of this study clearly indicated that *Codiaeum variegatum* leaves have ample potential to inhibit two human and animal pathogenic bacteria as seen from its strong inhibition against tested organisms. *Codiaeum variegatum* shows much promise in the development of phyto-medicine

having antimicrobial properties and the drug derived from *Codiaeum variegatum* may have the possibility of alternative medicinal source because of their antibacterial activity. This study also indicated that ethanol and water extract of this plant has highest capability to antibacterial activity against *Serratia marcescens* and *Bacillus subtilis*. In general, it can be concluded that ethanol and water extracts of *Codiaeum variegatum* is a strong inhibitor for bacterial growth. Therefore, it is recommended to identify the active ingredients of the antibacterial agent and obtaining chemotherapist agent in different drug formulations therefore be used for enteric and systemic infections caused by *Serratia marcescens* and *Bacillus subtilis.*

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التركيب الكيميائي والتأثير المضاد للبكتريا لأوراق نبات الكروتون *Codiaeum variegatum*

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المركبات الطبيعية المستخرجة من أجزاء النبات المختلفة لها أهمية كبيرة في المجال العلاجي حيث يمكن استخدامها في العديد من المجالات مثل علاج العدوي الناتجة عن أنواع البكتريا الممرضة، تم دراسة التأثير المضاد للبكتريا للمستخلص الإيثانولي والمائي لأوراق نبات الكروتون علي الكائنات الدقيقة (بكتريا موجبه لجرام *Bacillus subtilis* وبكتريا سالبه لجرام *Serratia marcescens*)، وقد أشارت النتائج أن كلا المستخلصين الإيثانولي والمائي قد أعطوا نتائج جيدة ضد البكتريا الموجبة والسالبة لجرام، هذا ويمكن تطوير نتائج هذا النبات الطبي إلى منتجات عشبية قياسية بسيطة التكلفة وآمنة وقد يكون بمثابة مصدر للعديد من المركبات المضادة للميكروبات.

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