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MAA and HG designed the study. AA performed the biological experiments, AYM conducted chemical experiments. AA and AYM wrote and revised the paper. MAA approved the final draft for publication.

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Phytochemical and Bioactive Evaluation of *Cissus rotundifolia* and *Maydis stigma* Cultivated in Taiz, Yemen

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Abstract:

The aim of the study was to investigate antioxidant and antibacterial activity of methanolic leaf extracts of *Cissus rotundifolia* (CR) and *Maydis stigma* (corn silk) type Zea mays L from Taiz -Yemen. Phytochemical analysis of *Cissus rotundifolia* extract indicated the presence of triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, coumarins, and saponins, and the *Maydis stigma* (corn silk) type Zea mays L extract indicated the presence of alkaloids triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, saponins, proteins and free amino acids. The extracts were tested against different bacteria such as *Pseudomonas aeruginosa*, *Proteus* and *Klebsiella*. The methanol extracts of *Cissus rotundifolia* showed effective free radical scavenging in the DPPH assay.

INTRODUCTION

Plants have been an interesting source of medicine, and many components of plant products are specially targeted against resistant pathogenic bacteria (Ashraf *et al.*, 2020; Gumaih *et al.*, 2020; Iqbal and Ashraf, 2018; Nostro *et al.*, 2006; Shahzad *et al.*, 2017). The significance of herbal plants in curing diseases has been revealed by proteomics studies (Zaynab *et al.*, 2018).

Cissus rotundifolia (CR) belongs to the family vitaceae genus *Cissus* (Al-Fatimi *et al.*, 2007). This family consists of 14 genera and 850 species which are distributed in tropical and reaching temperate climates of mainly East Africa, Zimbabwe, Mozambique, South Africa, and parts of Central Africa, Egypt and the Arabian Peninsula (FAO, 1988). *C. rotundifolia* commonly known in Yemen alhals, alfaq which used as herbal product (Al-Mamary *et al.*, 2002). Boiling the leaves of *C. rotundifolia* is a common appetizer in Yemen, and it's also used as an antipyretic in the treatment of malaria and dengue fever (Ali *et al.*, 2015). *C. rotundifolia* has been mainly used in traditional medicine for malaria, gastrointestinal trouble, loss of appetite, skin disease, burns; young shoot are edible. Digestive, foodstuff, fever, liver diseases and otitis (Schopen 1983; Al-Fatimi *et al.*, 2007; Iqbal and Ashraf, 2020).

Biological studies reported the use of *Cissus rotundifolia* for treating malaria (Ali *et al.*, 2015), analgesic, anti-inflammatory, antiulcerative, antioxidant and hepatoprotective activity (Said *et al.*, 2015; Hegazy *et al.*, 2019), antibacterial (AL-Bukhaiti *et al.*, 2021) anti-diabetic activity (Shalabi *et al.*, 2018; Mohammed *et al.*, 2019), antihyperlipidemic (Shalabi *et al.*, 2018), hypoglycemic activity (Al-Mehdar and Albattah, 2016) and anti-fertility agent (Mziray *et al.*, 2020).

Phytochemical screening of *C. rotundifolia* leaves and stem extracts showed the presence of steroids, flavonoids, proteins, triterpenes, carbohydrates, and/or glycosides, tannins, coumarins, and saponins (Said *et al.*, 2015), β -sitosterol, Magnificol, β -sitosterol-Dglucoside,

Quercetrin, Linarin, Quercetin, Isoorientin and Vitamins A, C & E (Shalabi *et al.*, 2018; Hegazy *et al.*, 2019). A nutritional analysis of the herbal plant *C. rotundifolia* revealed that it contains essential amino acids, fat, protein, crude fiber and minerals (Magnesium, Sodium, Potassium, Calcium, Phosphorus, Iron, Zinc, Manganese, Copper, Chromium (Hegazy *et al.*, 2019).

Another plant *Maydis stigma* (corn silk) type *Zea mays* L, belonging to the family Gramineae. Literature survey of this plant revealed phytochemical constituents: terpenoids, Steroids, alkaloids, saponins, tannins, flavonoids flavan-3-ol, steroids, flavanones, proanthocyanins polysaccharides and proteins, vitamins, carbohydrates, salts of Ca^{2+} , K^+ , Mg^{2+} and Na^+ and volatile oils (Bhaigyabati *et al.*, 2011; Hu and Deng, 2011; Hasanudin *et al.*, 2012; Solihah *et al.*, 2012; Yuan *et al.*, 2018; Aourabi *et al.*, 2020; Duru, 2020). Plant is reported as anti-hypertensive (Li *et al.*, 2019), neuroprotective (Choi *et al.*, 2014), antibiotic (Waiss *et al.*, 1979), antitumor (Pan *et al.*, 2017), antidiabetic effects (Zhao *et al.*, 2012), antioxidant and antibacterial activities (Alam, 2011), anti-prostatitis and anti-spasmodic activities (Buhner, (2007), anti-fatigue activity (Hu *et al.*, 2010), diuretic (Velazquez *et al.*, 2005). Moreover, they may show inhibition of IgE formation (Namba *et al.*, 1993) and anticoagulant activity (Abdel-Wahab *et al.*, 2002).

To date, various commercial corn silk (CS) drugs for medical purposes are available in the market (Wan Rosli *et al.*, 2008). Recently dry silk extract of *Maydis stigma* (*Zea mays* L.) is used to synthesize NiO nanoparticles (NPs) (Nwanya *et al.*, 2020). Traditionally corn silk is used in Yemen for the treatment of kidney stones. The main aim of the present study was to carry out a proximate analysis, phytochemical screening, and bioactivities evaluation of *C. rotundifolia* and *M. stigma* (corn silk) type *Zea mays* L. leave extracts that growing in Taiz-Yemem.

MATERIALS AND METHODS

Collection of sample

Leaves of Halas; *Cissus rotundifolia* (CR) and Fresh hairs *Maydis stigma* (corn silk) type *Zea mays* L. were collected from Taiz governorate in Yemen and were identified and authenticated at a plant taxonomist at by Abdul Nasser Al- Gifri, Professor of Plant Taxonomy Dept. of Biology Sciences Faculty, Aden University of Yemen.

Preparation of extracts

Plants was carefully washed with tap water, rinsed with distilled water, chopped into small pieces and shade dried at room temperature, then they were grinding into fine powder. The 70% methanol extract of bioactive material from the powder was prepared according to (Said *et al.*, 2015). With slight changes by using soxhlet apparatus, the extract was concentrated by a rotary evaporator and subjected to freeze drying in a freezer.

Phytochemical screening

The extracts were prepared and analyzed for the presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides, proteins, amino acids, triterpenoids and carbohydrates according to the protocols in the literature (Solihah *et al.*, 2012; Pandey and Tripathi, 2014).

Test for Alkaloids

The extract was dissolve in dil.HCl and filtered. The filtrate was treated with equal amount of Dragondroffs' reagent. The appearance of orange precipitate indicated the presence of alkaloids.

Test for Saponins

Five ml of distilled water was mixed with one ml of extract in a test tube and shaken for 5 minutes. Frothing was observed as the indicator for saponins.

Test of Tannins

One ml of extract was treated with 3% FeCl₃. Formation of greenish black precipitate indicates the presence of tannins.

Test of Flavonoid

One ml of extract was treated with 10% lead acetate. Formation of a muddy brownish precipitate after shaking indicates the presence of flavonoids.

Test for Coumarin

One ml of extract was treated with 10% NaOH. Formation of yellow colour shows the presence of coumarin.

Test of cardiac glycosides

0.2 g of extract was dissolved in pyridine, then added sodium nitroprusside solution and 10% NaOH. Formation of deep red colour indicated the presence of a deoxysugar characteristic of cardioids.

Test for Proteins

To 2 ml of extract solution, few drops of conc. HNO₃ were added. Appearance of yellow colour indicated the presence of proteins.

Test for Amino Acids

Two ml of extract was treated with 2% of Ninhydrin reagent and boiled in water bath for 20 minutes. Formation of purple colour indicated the presence of free amino acids.

Test for Tri-Terpenoids

0.2 g of extract was dissolved in chloroform and filtered. The filtrate was treated with conc. H₂SO₄, shaking and let to stand. Formation of golden yellow colour indicates the presence of triterpenes.

Test for reducing sugars

Two ml of extract was treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicates the presence of reducing sugars.

Free radical scavenging activity using DPPH assay.

The antioxidant activity of the methanolic extracts was assessed by measuring their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals compared to ascorbic acid as a standard. Radical scavenging activity of plant extract against (DPPH) was determined at wave length 517 nm on a UV visible light spectrophotometer. 3 ml of freshly prepared methanolic DPPH solution (6×10^{-5} M) were mixed with 100 µg/ml concentration of the plant extract. The samples were kept in the dark for 15 mins at room temperature then the UV absorbance was measured. The measurements were repeated in triplicate (Pal *et al.*, 2011).

Radical scavenging activity was calculated by the formula

$$\% \text{ Inhibition} = [(A_B - A_A) / A_B] \times 100$$

Where A_B = absorption of blank sample (t= 0 min)

A_A = absorption of test extract solution (t=15 mins)

Antibacterial activity

Three bacterial strains were used for the study. Gram negative bacteria include *Pseudomonas aeruginosa*, *Proteus* and *Klebsiella pneumonia*. All the tested strains were local isolates obtained from Department of Biology, Division of Microbiology, Faculty of Science, Sana'a University. These bacteria served as test pathogens for antibacterial activity assay. Three different concentrations of each extract of selected plants (50, 100 and 150 mg/ml) were dissolved in 10% dimethylsulfoxide (DMSO) in purified water to be used in antimicrobial activity test. Extract solutions were prepared just before carrying out the test.

The bacterial suspensions containing 10^6 CFU/ml (The bacteria prepared as suspension which contain 10^6 CFU/ml) of bacteria were spread on petri dishes plates with a sterile swab moistened with the bacterial suspension. In each

of these plates, five wells were cut out using a standard corn borer (7 mm). About 60µl of each extract was added into different wells (duplicate each concentration), dimethyl sulfoxide DMSO was used as a negative control. Positive control antibiotic wells were placed in the plate. All the plates were incubated at 37°C for 24hrs. After incubation bioactivity was evaluated by measuring the zone of inhibition. The experiment was performed in one of antibiotics standard. Neomycin was used as reference to determine the sensitivity of each bacterial species tested and used as control positive. The antibacterial activity of *C. rotundifolia* and Corn silk (*M. stigma*) extract was determined by agar well diffusion method according to previous studies (Hussain *et al.*, 2016; Ma *et al.*, 2018) with slight modifications.

Statistical Analysis

The data were expressed as a mean \pm SE. The statistical significance between groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey Multiple Comparisons methods using Prism 6 (Graph Pad, San Diego, CA, USA) at $p < 0.05$.

RESULTS

Phytochemical screening of plant materials

The phytochemical active compounds of *Cissus rotundifolia* and *Maydis stigma* (corn silk) type *Zea mays* L. were qualitatively analyzed and the results are presented in (Table 1).

Phytochemical analysis of 70% methanol extract of *C. rotundifolia* non-flowering aerial parts indicated the presence of alkaloids, triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, Coumarin and saponins, whereas the proteins and free amino acids were absent. On the other hand, 70% methanol extract of *M. stigma* (corn silk) indicated the presence of alkaloids triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, saponins, proteins and free amino acids, whereas coumarins were absent.

Free radical scavenging activity (DPPH assay) of *C. rotundifolia* and *M. stigma* extract

This method is based on the reduction of the stable free radical diphenylpicrylhydrazyl (DPPH) in the presence of an antioxidant to the non-radical form of yellow colored DPPH-H. Mean regarding DPPH scavenging activity of *C. rotundifolia* and *M. stigma* are presented in

(Table 2) and (Figure 1). The free radical scavenging activities of ascorbic acid was higher (93.89%), followed by *C. rotundifolia* (82.37%) and *M. stigma* (80.58%).

The free radical scavenging activity of *C. rotundifolia* and *M. stigma* extracts in contrast with ascorbic acid (As A) as standard antioxidant showed a statistically significant difference at $p < 0.05$.

Table 1. The qualitative test for preliminary phytochemical analysis of *Cissus rotundifolia* and *Maydis stigma*.

Sr. No.	Phytochemical analyzed	<i>Cissus rotundifolia</i>	<i>Maydis stigma</i>
1	Alkaloids	+	++
2	Saponins	++	+
3	Tannins	+	++
4	Flavonoids	++	+
5	Coumarin	+	-
6	cardiac glycosides	+	+
7	Proteins	-	+
8	Amino Acids	-	+
9	Tri-terpenoids	+	++
10	Reducing Sugar	+	+

(+) present; (-) absent; (++) appreciably present.

Table 2. Free radical scavenging activity of the CR and CS.

Parameters	DPPH (%)
Ascorbic acid	93.89 ± 4.842
<i>C. rotundifolia</i>	80.58 ± 1.840
Corn silk (<i>Maydis stigma</i>)	82.37 ± 3.055

Values are expressed in mean ± SE of 3 times repeated for each set of *C. rotundifolia* and *M. stigma* extracts

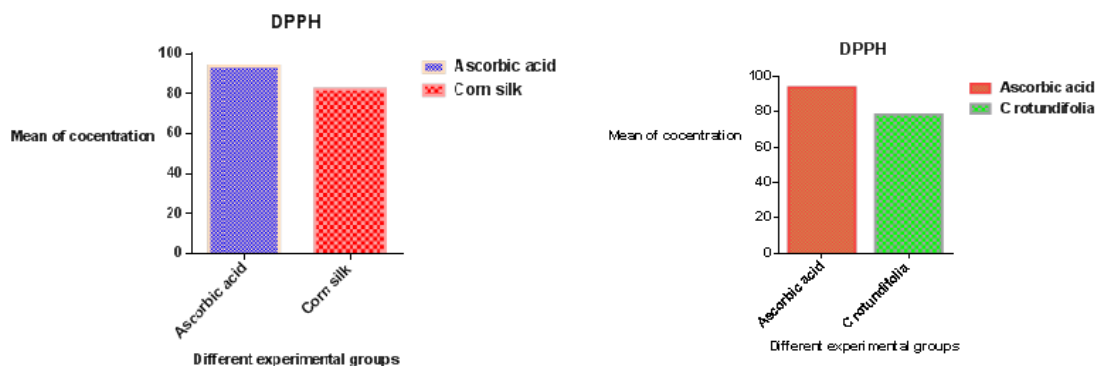


Fig. 1. Free radical scavenging activity of the *C. rotundifolia* and *M. stigma* extracts.

Antibacterial activity

The extracts of *C. rotundifolia* and *M. stigma* (corn silk) were tested in different concentrations (150mg, 100mg and 50mg) for antibacterial

effect against three kinds of bacteria (*Pseudomonas aeruginosa*, *Proteus* and *Klebsiella pneumonia*). The samples of these extracts and the dilution were plated on agar

plates to determine the antibacterial activity by measuring the diameter of the inhibition zone around the wells.

C. rotundifolia extract showed the lowest inhibition zone against *Klebsiella pneumonia*. The activity against *Klebsiella* was very low at a concentration of 50mg/ml and highest at a concentration of 150mg/ml. *M. stigma* (corn silk) extract showed high antibacterial activity with *Pseudomonas aeruginosa*. The activity against *P. aeruginosa* was very low at a concentration of

50mg/ml and the highest at a concentration of 150mg/ml. The zones of growth inhibition for all bacteria that were used were shown in (Table 3). All inhibition zones of these bacteria were compared with standard using Neomycin antibiotics, the results showed that the diameter of the inhibition zone was 14, 15, 16 and 17mm with antibiotics. The Placebo was a 10% DMSO solution and used as a negative control that revealed no inhibition zone with all tested organisms.

Table 3. Antibacterial activity of the CR and CS.

Organisms	Plant extracts	Conc & Zones/ mm			Standard	Plac*
		50 mg	100mg	150 mg	Neomycin	
<i>Klebsiella pneumonia</i>	<i>Cissus rotundifolia</i>	5mm	7mm	9mm	17mm	0
	<i>Maydis stigma</i>	12mm	15mm	22mm	16mm	0
<i>Pseudomonas aeruginosa</i>	<i>Cissus rotundifolia</i>	10mm	13mm	14mm	14mm	0
	<i>Maydis stigma</i>	6mm	20mm	24mm	14mm	0
Proteus	<i>Cissus rotundifolia</i>	15mm	16mm	19mm	15mm	0
	<i>Maydis stigma</i>	12mm	15mm	18mm	14mm	0

DISCUSSION

The present study revealed the presence of medicinally active constituents in *Cissus rotundifolia* and *Maydis stigma* (corn silk) extracts. The phytochemical screening of *C. rotundifolia* extract indicates the presence of alkaloids, triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, coumarin and saponins. Proteins and free amino acids were absent in these extracts. These results are in agreement with earlier works (Hegazy *et al.*, 2019; Mziray *et al.*, 2020) with the exception that our 70% methanol extract contain alkaloids whereas (Said *et al.*, 2015) who used 70% methanol by soaking method, no alkaloids were found. On the other hand *M. stigma* (corn silk) extract indicated the presence of alkaloids triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, saponins, proteins and free amino acids. Coumarins were absent. These results are in agreement with previous studies (Bhaigyabati *et al.*, 2011; Hasanudin *et al.*, 2012; Solihah *et al.*, 2012; Aourabi *et al.*, 2020).

Antioxidants are free radical scavengers, which provide protection to living organism from damage caused by free radicals inducing by environmental elements (Sen *et al.*, 2010).

Medicinal plants such as *C. rotundifolia* and *M. stigma* have a wide variety of phenolic compounds such as flavonoids that act potentially as antioxidants, scavenging reactive oxygen species (ROS) and inhibit LPO (Al-Mamary, 2002; Bhaigyabati *et al.*, 2011). Corn extracts have been reported to improve the antioxidant status of various organs by affecting the activity of antioxidant enzymes (Hu and Deng, 2011).

Phytochemicals are the non-nutritional bioactive compounds found in various parts of plants. These compounds are also important in pharmaceutical and medicinal field due to their antioxidant, antimicrobial, and other biological properties (Hasanudin *et al.*, 2012; Iqbal and Ashraf, 2019a,b,c).

The flavonoids found in this study belong to an important natural metabolite group with effective modulatory potential having antimicrobial potential (Flambó, 2013).

To our knowledge searching for herbal resources owning antimicrobial activity have been increased due to their possibility uses in several chronic and infection diseases. Among famous medicinal plants used in Yemen, our current study used 70% methanol extracts of *C. rotundifolia* and *M. stigma* to test these plants against gram negative bacteria such as *Pseudomonas aeruginosa*, *Protest* and *Klebsiella* sp. This study showed that the highest antimicrobial activity was with the higher concentration of 150mg/ml and low antibacterial activity at concentration of 50mg/ml for both extracts. Neomycin was used as standard to compare between samples and standard.

Our results were in agreement with previous studies that showed high antimicrobial activity (Nessa *et al.*, 2012; Emmanuel *et al.*, 2016; AL-Bukhaiti *et al.*, 2021). Tannins found in the present extracts have the ability to form protein complexes capable of controlling fungi and bacteria (Nishizawa *et al.*, 1990). The methanol fraction showed moderate activity to scavenge DPPH radical with IC_{50} $80.58 \pm 1.840 \mu\text{g/ml}$. These results were in agreement with the study reported (Shalabi *et al.*, 2018).

CONCLUSION

In conclusion, the results of the present study support the idea that medicinal plants as *Cissus rotundifolia* (CR) and *Maydis stigma* (corn silk) type *Zea mays* L from Taiz -Yemen can be promising sources for potential antibacterial and antioxidant agents. These results also form the basis for selection of plant species for further investigation in the discovery of new natural bioactive compounds. Thus, the study has shown the path that traditionally used easily available weeds can be a low-cost source of important bioactive molecules with potential for herbal drug development.

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CONFLICT OF INTEREST

There is no conflict of interest.

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