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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *COLOCASIA ESCULENTA* (TARO) MEDICINAL PLANT LEAVES USED IN FOLK MEDICINE FOR TREATMENT OF WOUNDS AND BURNS IN HUFASH DISTRICT AL MAHWEET GOVERNORATE–YEMEN

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ABSTRACT
In this study methanolic and aqueous extracts of one plant namely *Colocasia esculenta*, were screened for the presence of phytochemical constituents and tested for their antimicrobial and antioxidant activity. The qualitative phytochemical analysis revealed the results showed presence of alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in leaves plant. TLC tests conducted revealed Rf values in the leaves for alkaloids, flavonoids, tannins, phenols and saponins (0.95-0.96-0.97-0.96-0.97) respectively. The antimicrobial activity extracts against four bacterial isolates *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and Klebsiella sp. and a single fungal isolate *Candida albicans* with concentrations (0.5 mg/ml, and 1,0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control. The antioxidative activity of leaf was evaluated by using 1,1- diphenyl-2 picrylhydrazyl (DPPH), the results showed are 86.5%, lowest from standard, ascorbic acid 87.5%.
Keywords: Antimicrobial, antioxidative, *Colocasia esculenta*, phytochemical.

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INTRODUCTION

Colocasia esculenta (CE) Linn. (Family: Araceae) is an annual herbaceous plant with a long history of usage in traditional medicine in several countries across the world, especially in the tropical and subtropical regions. The herb has been known since ancient times for its curative properties and has been utilized for treatment of various ailments such as asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders, and skin disorders. The juice of CE corm is widely used for treatment of body ache and baldness[1]. *Colocasia esculenta* is phytochemically, these also contain flavones, apigenin, luteolin , and anthocyanins[2]**.** In a study, it was found that the most common isolates were *K. pneumoniae* (34.40%), followed by *P. aeruginosa* (23.94%), *S. aureus* (22.94%), *E. coli* (7.34%), *Acinetobacter* species (2.75%), *P. mirabilis* (2.75%), *Citrobacter* species (1.38%), and *Candida* species (4.59%)[3]. Methanol extract of *Colocasia esculenta* leaves has shown higher antioxidant activity81.77%[4].

MATERIALS AND METHODS

Sampling

The Samples of 100g of the grinded powder were put in sterilized flasks together with 400 ml of pure methanol for methanolic extraction treatments, while for aqueous extraction treatments, samples of 100g of grinded powder were put in sterilized flasks with 400 ml of distilled water each. All flasks were covered with transparent nylon and tin and then all were put on a rotary shaker machine for 24 hours, the speed of the device was 200rpm at the laboratory temperature (22.7^{o}C). The filtration process for each sample was

carried out using filter paper to obtain a pure solution. The evaporation process for each methanol solution and distilled water was conducted separately in the evaporator (methanol solution at 42°C and pressure 337. The distilled water solution at 45° C and pressure 72 for 2 hours for methanol solution and 4 hours for distilled water solution. Then obtained extracts were kept in dark conditions in the refrigerator at 4°C until used in the experiment[5].

Qualitative tests

Phytochemical screening of plant extracts

The methanolic and aqueous extracts subjected to phytochemical screening were alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acids[6,7].

Alkaloids: Dragendorff's test

In a test tube, 2-3 drops of Dragendorff's reagent was added to 0.1 ml of the extract orange precipitate indicated the presence of alkaloids.

Terpenoids: Salkowski test

In a test tube 5ml of extract was mixed in 2 ml of chloroform and then 3 ml of concentrated sulfuric acid was added to form a layer. A reddish brown coloration forms at interface.

Glycosides: Keller-Killani test

Concentrated sulfuric acid in a test tube and extract sample were mixed with glacial acetic acid containing 1 drop of Ferric chloride (1:1:1 volume). A brown ring appears in the presence of glycosides.

Resins: Turbidity test

To 5ml extract 5ml distilled water was added, the occurrence of turbidity shows the presence of resins.

Saponins: Foam test

A 5ml extract was shaken with 2 ml of distilled water. If foams are produced and persists for ten minutes this indicates the presence of saponins.

Tannins: $Fecl_3$ test

A 4ml extract was treated with 4ml $FeCl_3$, the formation of green colour was taken as positive for tannin.

Flavonoids: Shinoda test

Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

Phenols: $Fecl_3$ test

Extract was mixed with 2 ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols.

Amino acids: Biuret test

Extracts and 1 drop 2% Copper sulphate solution and 1 ml 95% ethanol excess of potassium hydroxide were mixed. Pink or yellow color in ethanol layer appears.

Thin Layer Chromatography

One gram of *Colocasia esculenta* powder was boiled with of with solvent system made from 15ml H_2SO_4 test for Alkaloids, 10ml 70% ethanol test for Flavonoides and Saponins, 25 ml water test for Tannins and Phenols15ml H_2SO_4 test for Alkaloids in rounded flasks. The TLC plate was prepared as such: (Layer: silica gel layers 0.25 mm thickness, 10 cm length and 5cm wide). The filtrate obtained was evaporated to dryness in a water bath at 37° C .The residue was dissolved by 0.2ml methanol. The solution was used for spotting the TLC by capillary tube by only one centered spot. The TLC plate was put inside a saturated tank, and development was waited. When the mobile phase reaches two thirds of plate's length, the plate was lifted out from the tank and let to dry in air. The plate was examined by U.V. lamp at the wavelength 365nm. The colors of florescence appeared and recorded. The plate was sprayed carefully reagent, and let to dry for 10 min then sprayed with solution. After it plate was examined under U.V. lamp at the wavelength 365nm. The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f values) of the various spots was calculated[6]. TLC was performed for alkaloids, flavonoids, tannins and phenols solvent system and confirmatory tests are shown in Table 1. Calculation of RF of each spot was as follows:

$$R_F = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

Antimicrobial activity of plants extracts

Microbial Cultures: Fresh plates of the four bacterial isolates *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella sp.* and a single fungal isolate *Candida albicans* were obtained from the National Center of Public Health Laboratories, Sana'a.

Media Use: The bacterial test were *spread* over the nutrient agar (56g/1000ML distilled Water) was weight into separate flask and dispensed into distilled water make a total volume of 1 liter. Then the fungal test were spread over the sabouraud dextrose ager (65g/1000ML distilled Water) was weighted into separate flask and dispensed into distilled water to make a total volume of 1 liter. These powders were dissolved in distilled water and used for evaluation of their antibacterial and antifungal activities. The mixture was heated in an electric water bath (GFC, 1083, Germany) until the Agar melted to form a homogenous solution. The prepared medium was separately transferred to Durum medium bottle and sterilized by autoclaving at 121°C for 30 minutes. The sterile medium was allowed to cool to about 45°C before being poured aseptically in an inoculation chamber (Ceslab England) in15 ml portions, into sterile petri dishes to cool and gel into solids[8].

Antimicrobial activity assay: Two different concentrations (0.5 mg/ml, and 1.0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control.

Zone of Inhibition

The bacteria plates were incubated at 37°C for 24hrs while the fungal plates were incubated at for 72 hours, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded.

Determination of antioxidant activity

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method used in a previous study[9]. The leaf extracts (20µl) were added to 0.5ml of methanolic

solution of DPPH (0.3mM in methanol) and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol, without the leaf extracts, Served as the positive control. After 30 min of incubation, the discolouration of the purple colour was measured at 517 nm in a spectrophotometer**)**. The radical scavenging activity (RSA100%) was calculated as follows:

Radical Scavenging Activity (RSA100%)= = $\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} X100$

Statistical Analysis

Analysis of variance was made for all data using (SPSS) version (25) computer program.

RESULTS AND DISCUSSION

In this study methanolic and aqueous extracts of one plants namely *Colocasia esculenta*, were screened for the presence of phytochemical constituents and tested for their microbial and antioxidant activity.

Yield from different solvents

Yield of methanolic extract of *Colocasia esculenta*, extracted with 100% methanol produced 29.14 (g). While yield of distilled water extract of *Colocasia esculenta* produced 26.45 (g).

Mean values of the yield are presented as mean ± SEM. Values are statistically significant when $p \leq 0.05$.

A similar investigation done in a study[10] newreferene stated that leaves of *Colocasia esculenta* gave 6.2%yeild when extracted with methanol, a far less amount than our findings (29.14%) while another study[11] estimated a 50% yield in aqueous extracts of *Colocasia esculenta* leaves. which is nearly double the amount found in this study[12]**,** as well as many authors attributed the variation in yield percentages to the extraction method as well as solvent composition.

Phytochemical composition of the methanolic and aqueous leaves extracts

The summarized phytochemical screening of chemical constituents of *Colocasia esculenta* extract is shown in Table 3. The results revealed the presence of active compounds in the two different extracts.

As the table shows, the methanol and aqueous extracts indicate the presence alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in all three plants .

In a qualitative phytochemical screening of *Colocasia esculenta* tubers methanolic and aqueous extract showed that alkaloids, glycosides, flavonoids, terpenes, saponins and phenol are present.

The results also showed the absence of tannins in both the extracts[13]. Additionally, a study[14] demonstrated that *Colocasia esculenta* leaves had a wide range of phytochemical compounds including flavonoids identified by phytochemical and analytical studies.

All previous findings were in harmony with our findings.

Thin Layer Chromatography (TLC)

Five secondary metabolites (alkaloids, flavonoids, tannins, phenols and saponins) were used for (TLC) thin layer chromatographic analysis.

Concerning *Colocasia esculenta*[15], in a study using thin layer chromatographic separation of methanol extracts gave three spots each with Rf values ranging from 0.60 – 0.70 these results were less than of this investigation. RF values of tubers of *Colocasia esculenta* in TLC analysis were low, in methanol extract (0.57-0.8) and in aqueous extract (0.51-0.52)[16], compared to RF higher values in methanol extract (0.96-0.97) and in aqueous extract (0.51-0.52) of leaves of *Colocasia esculenta* of the present study. This supports the fact that phytochemical constituents are more in quantity in the leaf parts of the plant.

Antibacterial and antifungal activity of plants extracts.

Antimicrobial activity of standard antibiotics discs against tested bacterial and fungal are shown in Table 8, Figure 1. The results of the study indicated that control Antibiotics against bacteria and Fungi showed different inhibitory zones. Antibiotics activity of AM (10ug), CIP (25ug), CF (30ug), PZ (75ug) and PC (100ug) against *Staphylococcus aureus were* 19, 26, 20, 21, 20 mm; *E. coli* 17, 28, 18, 20, 19 mm; *Pseudomonas aeruginosa* 18, 30, 17, 21, 18 mm; *Klebsilla sp*. 20, 33, 22, 23, 17 mm, and *Candida albicans* 21, 31, 20, 19, 22 mm respectively.

The microbial activity of the methanolic extracts of *Colocasia esculenta* against *Staphylococcus auerus* and *Escherichia coli* gave a higher inhibition zone compered to antibiotics except CIP. However, lower values were recorded with all antibiotics against Pseudomonas *aeruginosa* and *Klebsiella. sp*. except close values to CF and PC respectively. Accordingly both extracts showed lower effects against *Candida albicans* than all antibiotics used Table 5.

The microbial activity of the aqueous extracts of *Colocasia esculenta* against *Staphylococcus. aureus* and *Escherichia. coli* Table 6 gave lower diameters in inhibition zonescobaring with all standard antibiotics with the except of AM with *E. coli* which gave same value. However, higher values were recorded than all antibiotics against *P. aeruginosa* except CIP and PZ. On the other hand both extracts showed lower effects against *Klebsiella sp*. and *Candida albicans* than all other antibiotics. A study[17] explained that the leaves of *C. esculenta* extracted using distilled water showed antimicrobial activity against all the 5 strains of *Vibrio* spp. In the present study it was observed that that the extracts of *C. esculenta leaf,* extracted using distilled water, showed antimicrobial activity against all the tested bacterial isolates Table 6[18]. In study the methanolic aqueous extract at (50,100 mg) concentration inhibited *Staphylococcus aureus, E. coli* (50, 100mg) concentration inhibited *Staphylococcus aureus, E. coli*, *Pseudomonas aeruginosa*, (16, 10, 10mm) and (20, 13, 11mm) respectively[19]. In study the methanoli extract at (50, 100) mg concentration inhibited *Staphylococcus aureus* (11, 14mm). *E. coli* (8, 11mm), *Pseudomonas aeruginosa* (10, 14mm) *Klebsiella sp* (8, 11mm)[14]. In study the methanoli and

aqueous extract at100 mg concentration inhibited *Staphylococcus aureus* (10, 7mm). *E. coli* (8, 7mm), *Pseudomonas aeruginosa* (0, 0mm) *Klebsiella sp* (10, 11mm).

Antioxidant activity

Results showed are 86.5%, lowest from standard, ascorbic acid 87.5% (Table 7 and Figure 1)**.**

Methanol extract of *Colocasia esculenta* leaves has shown higher antioxidant activity 81.77%[4].

CONCLUSION

The present study showed that *Colocasia esculenta* are rich sources of useful secondary metabolites, It is strongly recommended of using them for general medicinal purpose and specially for treat wounds and burns diseases. It is strongly recommended of using them for production of effective pharmaceutical compounds and can be used as natural products of antimicrobial to treat wounds and burns diseases instead of chemical drugs. It is noticeable that the leaves of *Colocasia esculenta* are very rich in antioxidant content and therefore are good sources and safe and cheap for that .

CONFLICT OF INTEREST

"No conflict of interest associated with this work".

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Table 1: R_f values of TLC solvent system for different extracts of *Colocasia esculenta*.

Phytochemical	Mobile phase	Confirmatory test	Extract	R_F Value
Alkaloids	Acetone:water:26% ammonia (90:7:3)	Dragendorff reagent	1 ml HCL+ 9ml water	0.96
Flavonoides	Chloroform: Ethyl acetate (6:4)	Aluminum chloride reagent	70% ethanol	0.97
Tannins	Chloroform: Ethyl acetate (6:4)	10% $FeCl_3$ reagent	25ml water	0.99
Phenols	Toluene: Acetone: Formic acid (60:60:10)	10% KOH reagent	Methanol	0.97
Saponins	Ethyl acetate	Vanillin sulfuric acid reagent	Methanol	0.99

Table 2: Yields of *Colocasia esculenta* leaves extracts from methanolic and aqueous extracts.

M	Powder of plants	Amount of samples used (g)	Solvent	Volume of the solvent used (ml)	Extract yield/(g)*
1	*Colocasia esculenta*	100	Pure Methanol	400	29,14±0.07
2	*Colocasia esculenta*	100	Distilled water	400	26,45±0.06

Mean values of the yield are presented as mean±SEM. Values are statistically significant when p≤ 0.05.

Table 3: Phytochemical composition of the methanolic and aqueous leaves extracts of *Colocasia esculenta*.

Solvents	Chemical compounds								
	Alkaloids	Terpenoids	Glycosides	Resins	Saponins	Tannins	Flavonoids	Phenols	Amino acids
Methanolic extract	+	+	+	+	+	+	+	+	+
Aqueous extract	+	+	-	+	+	+	+	+	-

Absence (+) Presence (-)

Table 4: Antimicrobial activity of standard antibiotics discs against tested bacterial and fungal.

Organisms	Inhibition zones diameter (mm) of tested antibiotic				
	AM (10ug)	CIP (25ug)	CF (30ug)	PZ (75ug)	PC (100ug)
Staphylococcus aureus.	19	26	20	21	20
Escherichia coli.	17	28	18	20	19
Pseudomonas aeruginosa.	18	30	17	21	18
Klebsiella sp.	20	33	22	23	17
Candida albicans.	21	31	20	19	22

AM=Amoxycillin, CIP= Ciprofloxacin, CF=cefazllin, PZ=Cefoperazone, PC=piperacillin.

Table 5: Antimicrobial activity of the methanolic extracts of leaves of (*Colocasia esculenta*) and standard antibiotics discs against tested bacterial and fungal.

Organisms	Zone of inhibition(mm) Antibiotic						
	0.5 g/ml	1.0 g/ml	AM (10ug)	CIP (25ug)	CF (30ug)	PZ (75ug)	PC (100ug)
Staphylococcus aureus.	23	21	19	26	20	21	20
Escherichia coli.	20	21	17	28	18	20	19
Pseudomonas aeruginosa.	17	16	18	30	17	21	18
Klebsiella sp.	17	16	20	33	22	23	17
Candida albicans.	13	14	21	31	20	19	22

Table 6: Antimicrobial activity of the aqueous extract of leaves (*Colocasia esculenta)* and standard antibiotics discs against tested bacterial and fungal.

Organisms	Zone of inhibition(mm) Antibiotic						
	0.5 g/ml	1.0 g/ml	AM (10ug)	CIP (25ug)	CF (30ug)	PZ (75ug)	PC (100ug)
Staphylococcus aureus.	12	15	19	26	20	21	20
Escherichia coli.	13	17	17	28	18	20	19
Pseudomonas aeruginosa.	16	20	18	30	17	21	18
Klebsiella sp.	13	12	20	33	22	23	17
Candida albicans.	12	15	21	31	20	19	22

Table 7: Antioxidant activities of the selected extracts and L- ascorbic acid using the (DPPH) free radical-scavenging assay

Particular	Antioxidant activity DPPH (g/ml)
L- ascorbic acid	87.5±0.05
Colocasia esculenta	86.5±0.73

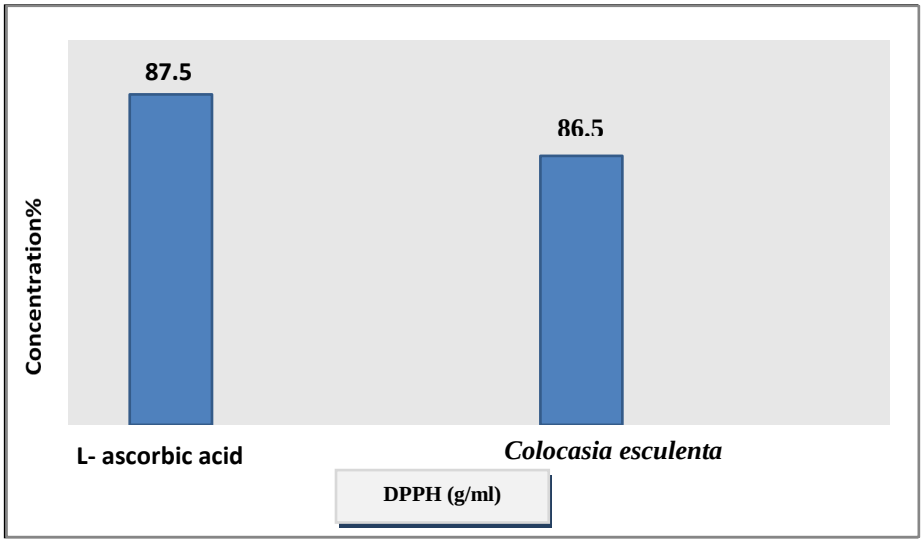

Figure 1: Antioxidant activities of the selected extracts and L- ascorbic acid using the (DPPH) free radical-scavenging assay.