Egypt. J. Chem. **Vol. 64,** No. 4 pp. 2165- 2172 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Phenolic Profile: Antimicrobial Activity and Antioxidant Capacity of Colocasia esculenta (L.) Schott.

Amani M. El-Mesallamy[a], Nader A. El-Tawil[b], Shereen A.Ibrahim[b], Sahar A.M. Hussein[c*]

[a]*Department of Chemistry, Faculty of Science, Zagazig University.*

[b]*Department of Nutrition , Central laboratory for Aquaculture Research, Cairo, Egypt*

[c]*Department of Phytochemistry and Plant Systematics,National Research Centre, Cairo, Egypt*

Abstract

The aim of the study was to investigate the presence of bioactive compounds in the ethanolic extract of *C. esculenta* by HPLC and GC methods. The identification of phytochemical compounds is based on the peak area, retention time and pharmacological actions. The HPLC chromatogram of ethanol extract showed the presence of 13 compounds namely, 6,2',4'-trimethoxyflavanone, benzoic acid, gallic acid, luteolin-6,8-C-diglucoside, catechin, chlorogenic acid , astilbin, quercetin, kampferol, and major compounds with high concentration of absorbance are , rutin, , vitexin, ellagic acid and caffeic acid. The GC analysis of petroleum ether of *C. esculenta* extract showed presence 31 saturated fatty acid ,volatile organic hydrocarbons and sesquiterpenes .The major components were determined as follows, saturated fatty acid, Stearic acid, Arachidonicacid, 8-Heptadecene, monounsaturated fatty acid, (S)-citronellic acid and volatile organic hydrocarbons, specifically, sesquiterpenes as D-Germacrene and Isoledene, The antimicrobial activity of ethanol extract showed characteristic zone of inhibition against six clinical pathogens. The highest zone of inhibition was observed at 100mg/ml concentration against , the (Gram Negative Bacteria) *Proteus vulgaris* (ATCC13315) at 15mm, while *Escherichia coli* (ATCC25955), at 13 mm and *Pseudomonas aeruginosa* (ATCC27853) at 12 mm , while the zone of inhibition against (Gram positive Bacteria) *Staphylococcus aureus* (RCMB010010), at 14 mm, *Enterococcus faecalis* (ATCC 29212), at 12 mm ,and no effect against *Bacillus subtitis* (RCMB 015 (1) NRRL B-543) , when compared with the standard drugs Gentamicin . The antifungal activity against six Fungi, was *Cryptococcus neoformans* (RCMB 0049001) at 16mm, *Aspergillus fumigatus* (RCMB 002008) at 11mm, *Syncephalastrum racemosum* RCM 016001 (1) at 10 mm, and *Candida albicans* (RCMB 005003 ATCC 10231) at 9 mm . The zone of inhibition was observed at 100 mg/ml concentration when compared with the standard drugs Ketoconazole with (MIC) 100 mg/ml . The antioxidant activities of the extracts were determined using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging . ethanol extract of *C. esculenta* exhibited with IC_{50} value (22 µg/mL), while the standard ascorbic acid with IC_{50} (11.4 µg/mL) .This result is promising . The above findings may justify the medicinal uses of the plants. The results suggest that the leaves of *C. esculenta* are a good sources of bioactive compounds with potent antimicrobial and antioxidant substances.

Keywords: *Colocasia esculenta*, bioactive , Phenolic compounds, profiling by HPLC, GC, Antibacterial, Antifungal, , Zone of inhibition, Antioxident

Introduction

The popularity of herbal medicine in recent times is based on the premise that plants contain natural substances that can promote health and alleviate illness. Herbal drugs or medicinal plants, and their extracts and isolated compounds have demonstrated a wide spectrum of biological activities **[1].** Taro is the common name of *Colocasia esculenta* Linn. (Family: Araceae) is an annual herbaceous plant and is a tropical and perennial plant. Taro is of great importance in many places such as the Philippines, Sri Lanka, India, Nigeria, Indonesia, Egypt and the eastern Mediterranean; **[2,3].** It is a well-known fact that traditional systems of medicines have always played important role in meeting the global healthcare needs. They are continuing to do so at present and shall play major role in future as well. To explore the medicinal importance of taro leaves. Traditionally, a decoction of the leaves is drunk to promote menstruation and

*Corresponding author e-mail: drsahar90@yahoo.com.; (*Sahar A.M. Hussein*).

Receive Date: 04 January 2021, Revise Date: 16 January 2021, Accept Date: 16 January 2021

DOI: 10.21608/EJCHEM.2021.56495.3213

together with other parts of the plants, it is used to relieve stomach problems **[4].** Therefore, the focus on plant research has increased all over the world and a large body of evidence show immense potential of medicinal plants used in various traditional folk medicine **[5].** Phytochemical analysis showed that the major constituents of *Colocasia esculenta* leaves are flavonoids β-sitosterol, and steroids, **[6,7].** The leaves of the plant are reported to possess huge vitamin C content. The pharmacological studies revealed that the plant exerted many pharmacological activities, including central nervous effects, antioxidant, anti-inflammatory, analgesic, anti-lipid Peroxidative activity, antidiabetic, antihepatotoxic and antimicrobial effects , antifungal activity due to presence of cystatin which has been reported to possess **[8,9].** Today, the ongoing emergence of multidrug-resistant bacteria and many diseases mainly caused by free radicals are serious global problems **[10,11]** . Thus, new antimicrobials and novel approaches to combating these problems are urgently needed. Combination therapy is a new approach that may be helpful in treating multidrug-resistant bacteria and diseases caused by oxidative stress **[12,13].**

This study was designed to highlight the phytochemical constituents of leaves extract of this plant was studied and analyzed by TLC, HPLC and GC and to determine pharmacological properties including antimicrobial, antioxidant properties of the ethanol , petroleum ether extracts of C. esculentus leaves.

The objective of this research also, to investigate antioxidant potential and the antimicrobial of *Colocasia esculenta* L. leaves. The free radical scavenging activity was measured using DPPH following the method of McCune and Johns **[14]**, as well as the antimicrobial activity against different microorganisms and their synergistic effects

Material and Methods

Plant Material

The leaves of *C. esculenta* L. were collected in April 2018 from the field. The plant was identified by Medicinal and Aromatic Plants department, Faculty of Science, Zagazig University. A voucher specimen has been deposited at the herbarium of Zagazig University. Egypt.

Plant Extraction

The leaves were cut into small pieces and dried at 40-45ºC , in an electric oven then grinded to produce 150 grams. The powder was defatted using a Soxhlet apparatus with petroleum ether (60-80). The defatted residue of plant was extracted exhaustively with ethyl acetate then by (5 × 3 liter) then finally with ethanol 95% till exhaustion. Each extract was concentrated under vacuum using rotary evaporator (Buchi R100) to yield dry extracts 28.52,20.40,35.26 gm, respectively.

Experimental :

General Section:

Paper chromatography was carried out on unwashed Whatman paper 1MM and preparative chromatography at 3MM, using two dimension solvent system AcOH-15 % and *n*-butanol:acetic acid:water, (BAW, 4:1:5 upper layer). Thin layer chromatography (TLC) was done to elucidate the components present in ethanol extract by its characteristics R_f values and UV lamp visualization (λ_{max}254 and 366 nm). UV spectral analysis, Shimadzu UV1700 spectrophotometer (Japan) and BUCHI , Rotavapor R-100 AG, (Switzerland) for solvent evaporation.

Chemicals:

All the chemicals and solvents used in this study were of pure and analytical grade. (DPPH), (1, 1-diphenyl-2-picryl-hydrazyl, Sigma-Aldrich Chemie, Germany). The standards chemicals like ascorbic acid, phenolic acids (gallic, caffeic, syringic, *p*-coumaric, ferulic, and sinapic), flavonoids (catechin, rutin, myricetin, quercetin, apigenin and kaempferol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the HPLC-grade solvents such as chloroform, methanol, water and acetic acid were purchased from Merck (Germany).

Analytical studies:

Thin Layer Chromatography For phytochemical screening:

The ethanol extract of *Colocasia esculenta* leaves was made in two dimensional paper chromatographic in BAW which used as mobile phase to separate the compounds according to their mobility. Separated spots were visualized under UV light and R_f factor was calculated by the following equation.

R_f factor = Distance travelled by solute front / Distance travelled by solvent .

The sprayed TLC after drying, some spots convert to dark blue colour which indicated to contain phenolic compounds.

Optimization of HPLC–DAD Conditions:

a) The analysis was performed on : Waters 2690 Alliance HPLC system equipped with a quaternary pump, an on-line degasser, an auto-sampler , and equipped with a Waters 996 photodiode array detector. b) Standard preparation: Mix of eight standards in 20 ml mobile phase then sonicated for 20 min , then filtered using 0.22 µm syringe filter then 10 µl were injected. c) Sample preparation: *C. esculenta* leaves ethanol extract filtered using 0.22 µm syringe filter then 10 µl were injected. d) HPLC analysis conditions:

The separation was carried out on a Zorbax SB-C_{18} column (4.6 mm×250 mm, 5 µm) . Mobile phase: Buffer (0.1 % phosphoric acid in water) and Methanol • Mode of elution: using gradient elution: 0–30 min (3%–100% B), There was a 5-min wash with 100% B after each run and equilibrium time was 15 min. Flow rate: 1ml/min • Temperature: Ambient • Wavelength: 254 nm

Gas Chromatography (GC) Analysis: GC Technique, was performed using a capillary column HP-5 (25 m × 250 µm i.d., 0.25 µm film thickness) in an Agilent 6890 gas chromatograph .The carrier gas was helium with a constant flow rate of 1 mL min–1. The oven temperature was initially kept at 50°C for 6 min then ramped at 4 °C min–1 to 300 °C and held isothermally for 30 min. Solutions of the samples (100 ppm in chloroform) were injected manually at 250 °C. Injection volume was 1.0 µL in the splitless mode.

Antioxidant testing assays

Evaluation of antioxidant activity by DPPH radical scavenging method

Radical scavenging activity of *C.esculenta* leaves extract against the stable free radical DPPH was determined spectrophotometrically . When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) using UV spectral data at λ_{max} 517 nm. Estimation was done to the method of **[15,16].** Where the extract solution was prepared by dissolving 0.025 g of the dry extract in 10 ml of methanol. The solution of DPPH in methanol (6×10^{-5} M) was freshly prepared, before UV measurements. This solution (1 ml) was added to 3 ml. of the extract in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). The resulting solutions were kept in the dark for 30 min at room temperature. The absorbance was measured at λ_{max} 517 nm. Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using following equation:

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

Where: AB is the absorbance of blank sample and AA is the absorbance of the tested samples

Evaluation of Antibacterial and Antifungal Activity:

Test organisms: The organisms used for the test were pure cultures of (Gram positive Bacteria) **:** *Staphylococcus aureus* (RCMB010010)**,** *Enterococcus faecalis* (ATCC 29212), *Bacillus subtitis* RCMB 015 (1) NRRL B-543**,** (Gram Negative Bacteria): *Escherichia coli* (RCMB 010052) (ATCC 25955), *Proteus vulgaris* RCMB004 (1), ATCC 13315, *Pseudomonas aeruginosa* (ATCC 27853). The antifungal atest against**:** *Aspergillus fumigatus* (RCMB 002008), *Syncephalastrum racemosum* RCM 016001 (1), *Candida albicans* (RCMB 005003) (ATCC 10231), and *Cryptococcus neoformans* (RCMB 0049001). These microorganisms were identified and confirmed by Microbiologists, in the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt, according to CLSI (2004), CLSI (2012). All bacteria and fungi were sub-cultured on nutrient agar and stored at 4°C until use

Preparation of plant extract impregnated discs:

Prepare discs of 5mm from Whatman paper no.1 Sterilization was done by autoclaving and dried at 70ºC for an hour in oven. Then the discs were impregnated with ethanolic extract of *C.esculenta.* Each produced disc has the ability to absorb about 0.01ml.

Testing method for Antibacterial and Antifungal activity:

Determination of antibacterial activity Bacterial susceptibility to antimicrobial agent was determined *in vitro* by using the standardized agar-disc diffusion method known as the Kirby Bauer method **[17].** The inhibition zone diameter was measured to determine antibacterial activity. Gentamycin sulfate and distilled water were used as positive and negative controls, respectively For each microbial strain negative controls were maintained where pure solvent DMSO was used instead of the extract since it does not possess any antimicrobial effect **[18]** and for positive control the standard antimicrobics Gentamicin 10 µg/disc for bacteria, The positive control used for fungi was ketoconazole . The plates were incubated overnight at 37˚C for bacterial strains and 42˚C for fungal strains. The experiment was performed under strict aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of inhibition. Three replicated agar plates were used for each different concentration and both controls (distilled water and 0.1% gentamycin sulfate). A total of 10 µl extract was added to a paper disc for each concentration and controls. Each disk was then placed in agar plate which had bacterial suspension in the plates. All plates were incubated at 37°C for 24 h. The diameter of inhibition zone created by each disc was measured (in mm) using a micrometer.

Results and Dissection:

Identification and quantification of different phenolic acids, flavonoids in EtOH extracts of *Colocasia esculenta*. by HPLC:

The HPLC chromatogram of ethanol extract of *C. esculenta* showed the presence of major compounds

namely, 6,2',4'-Trimethoxyflavanone, Benzoic acid, Vitexin, Gallic acid, Luteolin-6,8-C-diglucoside, Catechin, Chlorogenic acid , Caffeic acid, Rutin, Astilbin, Ellagic acid, Quercetin, Kampferol.
The major compounds with high concentration of absorbance are Rutin, Vitexin, Ellagic acid, Caffeic acid. **[Fig 1, 2, 3] and [Table 1]**

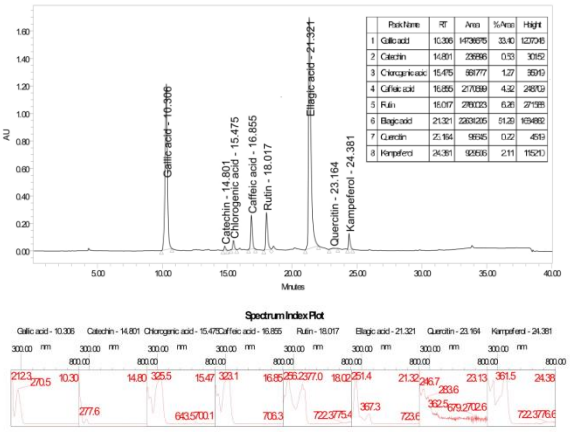


Fig.1: HPLC Chromatogram of standard , phenolic acids and flavonoids.1.Gallic acid, 2. Catechin, 3. Chlorogenic acid, 5.Caffeic acid, 6. Rutin,7. Ellagic acid ,8. Quercetin, 9. Kaempferol.

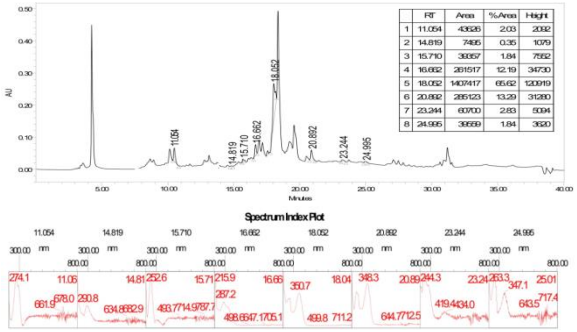
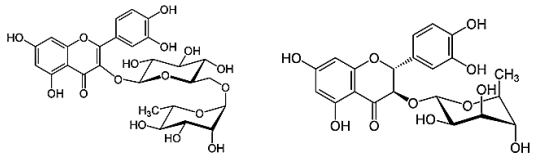


Fig 2: The HPLC chromatogram of ethanol extract of *C. esculenta* showed the presence of , 1.6,2',4'-Tri-methoxyflavanone, 2.Benzoic acid, 3.Vitexin, 4.Gallic acid,5.Luteolin-6,8-C-diglucoside, 6. Catechin, 7. Chlorogenic acid , 8.Caffeic acid, 9Rutin, 10.Astilbin, 11. Ellagic acid, 12. Quercetin, 13. Kampferol,

Table 1: Retention time and parameters of calibration curve, precision and repeatability, percent recovery study of standard phenolic acids and flavonoids for HPLC method validation.

No	Phenolic Compounds of *C. esculenta* leaves	$R_{t,\ min.}$	Area %
1.	6,2',4'-Trimethoxyflavanone	3.18	1.52
2.	Benzoic acid	8.84	5.14
3.	Vitexin	10.11	17.16
4.	Gallic acid	11.054	2.03
5.	Luteolin 6,8-C-diglucoside	12.6	5.24
6.	Catechin	14.819	0.35
7.	Chlorogenic acid	15.710	1.64
8.	Caffeic acid	16.662	9.59
9.	Rutin	18.052	24.42
10.	Astilbin	18.12	25.13
11.	Ellagic acid	20.892	10.59
12.	Quercetin	23.244	2.73
13.	Kampferol	24.995	1.84



Rutin Astilbin

Fig 3: Chemical structures of major favonoids in ethanol extract of *C. esculenta* leaves which have been identified by HPLC

The major compound rutin in **[Table 1],** reported to exhibit significant pharmacological activities, including anti-oxidation, anti-inflammation, anti-diabetic etc. It is also used for the treatment of vericose veins, haemorrhoids, haemorrhagic stroke and mucositis. So the leaves of *C. esculenta* might be considered as a good source of rutin and consumption of this plant as vegetable would be useful for health promotion. It is worthwhile to mention that the polarity increases the quantification of highly polar phenolic acids **[20],** and flavonoids like, rutin, quercetin and apigenin are detected in the extract . So the ethanol is found to be the optimum solvent of choice as it contains the maximum variety and the extent of bioactive components. Iwashina *et.,al*.1999, carried out isolation and identification of the flavonoids in the leaves of *C. esculenta* plant. The flavonoids were identified by UV spectral analysis. They isolated eight flavonoids *viz*. orientin, isoorientin, isovitexin, vicenin-2, orientin 7-*O*-glucoside, isovitexin 3'-*O*-glucoside, vitexin 2''-*O*-glucoside, luteolin 7-*O*-glucoside **[21]** . Among natural polyphenolics, kaempferol, is a flavonol found in many edible plants and is reported to possess potent pharmacological and nutraceutical activities. The consumption of plants containing kaempferol thereby conferring innumerable health benefits in the form of reducing scourge of cardio vascular diseases, cancer, arteriosclerosis etc. The antioxidant properties are

known to be responsible for these health benefits **[22].**

Identification and quantification of different volatile oil in petroleum ether extract of C. *esculenta*. By GC:

Gas Chromatography analysis of compounds was carried out in petroleum ether extract of C. *esculenta* , shown in **[Table 2]** .The GC chromatogram of the 31 peaks of the compounds detected was shown in **[Figure 4, 5].** The presence of 6 major peaks and the components corresponding to the peaks were determined as follows, saturated fatty acid as Stearic acid **,** Arachidonic acid, 8-Heptadecene, mono unsaturated fatty acidas (S)-Citronellic acid and volatile organic hydrocarbons, specifically, sesquiterpenes as D-Germacrene and Isoledene

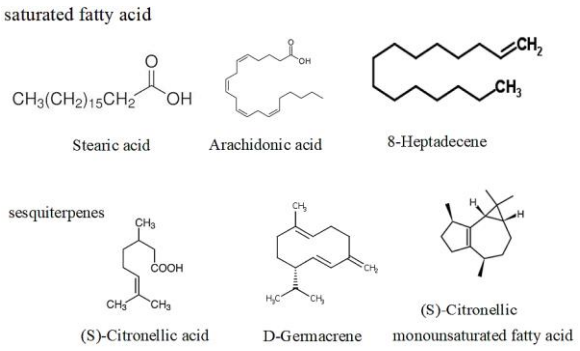


Figure 4 : Chemical structures of saturated fatty acid , volatile organic hydrocarbons, sesquiterpenes and monounsaturated fatty acid determined from petroleum ether extract of C. *esculenta*. By Gas Chromatograghy

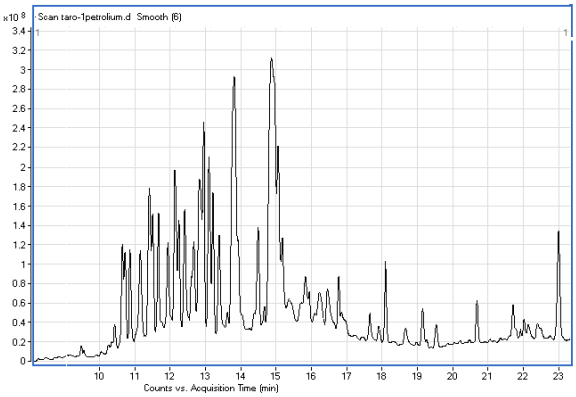


Figure 5 : Gas chromatography of volatile oil from petroleum ether extract of C. *esculenta*.

Table 2: Gas chromatography of volatile oil from petroleum ether extract of C. *esculenta*.

Peaks No	$R_{t(min)}$	Compounds	Area %
1.	10.6	β-Cubebene	2.67
2.	10.86	α-Gurjunene	2.06
3.	11.11	8-Heptadecene	3.63
4.	11.4	Isolongifolene	2.25
5.	11.6	β -Himachalene	2.37
6.	11.9	Cannabidiol dimethyl ether	2.07
7.	12.11	D-Germacrene	4.03
8.	12.23	Δ-Cadinene	2.28
9.	12.39	Ylangene	2.68
10.	12.65	Homomyrtenol	2.16
11.	12.8	Isoledene	3.11
12.	12.92	Linoleic acid	3.09
13.	13.08	Cis-thujopsene	2.71
14.	13.16	Phytol	1.94
15.	13.4	α-Muurolene	3.07
16.	13.7	Stearic acid	13.07
17.	14.5	Ethyl linolenate	2.01
18.	14.88	Arachidonic acid	14.33
19.	15.08	(S)-Citronellic acid	4.48
20.	15.9	B-Chamigrene	2.89
21.	16.12	Farnesol	1.6
22.	16.4	Geranyl isovalerate	1.33
23.	16.74	1-Heptadecene	2.16
24.	18.08	N-Butyric Acid 2-Ethyl Ester	1.21
25.	18.67	Stigmasterol acetate	1.94
26.	19.13	1-Octadecanol	0.90
27.	19.53	Z-2-Dodecenol	1.35
28.	20.93	Octacosene	1.24
29.	21.7	1-Eicosene	1.22
30.	22.4	Δ-Tocopherol	0.79
31.	22.99	Tetratetracontane	2.86

Antimicrobial study:

A significant part of the chemical diversity produced by plants protects them against microbial pathogens. The antimicrobial activity of *C. esculenta* in ethanol solvent was evaluated against 3 gram-positive bacteria, 3 gram negative bacteria, and 6 fungal strains. The highest zone of inhibition was observed at 100mg/ml

concentration against *Proteus vulgaris* at 15 mm, while ,against *S.aureus* at 14 mm , *E. faecalis* at 12 mm, *E. coli* at 13 mm , *Pseudomonas aeruginosa* at 12mm , and no effect against *B. subtitis* **,** when compared with the standard drugs Gentamicin. The highest zone of inhibition was observed at 100mg/ml concentration against Fungus , was *Cryptococcus neoformans* at 16mm, *Aspergillus fumigatus at* 11mm, *Syncephalastrum racemosum* at 10mm, and *Candida albicans* at 9mm when compared with the standard drugs Ketoconazole with (MIC) 100 mg/ml**.**
(Table 3, 4)

Table 3: Antibacterial Activity of Ethanolic Extract of Leaves of *C. Esculenta* By Measuring the Zone of Inhibition In Agar Disc Diffusion Method

Measurement of Zone of inhibition (in mm)						
	Gram positive Bacteria			**Gram Negative Bacteria**		
Bacteria	***Staphylococcs aureus*** **RCMB01000**	***Enterococcs faecalis*** **ATCC 29212**	***Bacillus subtitis*** **RCMB015 (1)NRRLB-543**	***Escherichia coli,*** **RCMB 010052 ATCC 25955**	***Proteus Vulgaris*** **RCMB004 (1), ATCC 13315**	***Pseudomonas aeruginosa*** **ATCC 27853**
Ethanol extract	14	12	NA	13	15	12
Gentamycin drug	24	20	26	30	25	27

Table 4: Antifungal Activity of Ethanolic Extract of Leaves of *C. Esculenta* By Measuring the Zone of Inhibition In Agar Disc Diffusion Method

Measurement of Zone of inhibition (in mm)				
FUNGI	***Aspergillus fumigatus*** **RCMB 002008**	***Syncephalastrum racemosum*** **RCM 016001 (1)**	***Candida albicans*** **RCMB 005003 ATCC 10231**	***Cryptococcus neoformans*** **RCMB 0049001**
Ethanol extract	11	10	9	16
Ketoconazole drug	17	15	20	25

Free Radical Scavenging Activity By DPPH:
The free radical scavenging activities of ethanol extract of leaves of C. esculenta were tested by the DPPH method. The IC_{50} values of DPPH free radical scavenging activity of ethanol extract of *C. esculenta* showed an IC_{50} value greater than 1000 µg/mL. The maximum scavenging activity with IC_{50} value (22 µg/mL), which was near that of standard ascorbic acid IC_{50} (11.4 µg/mL). This result is promising because it indicates a reduction in the concentration of the drug used in combination for treating various diseases and disorders.

Conclusion

The reversed-phase HPLC method with diode array detection was developed for the quantitative estimation of phenolic and flavonoids in EtOH extract of *C. esculenta*. The established HPLC assay showed a well separation of the compounds and also the developed method was linear, sensitive, accurate, meticulous and reproducible. Therefore, the method is suitable for the simultaneous determination of phenolic and flavonoids .

The presence of significant amount of respective bioactive components in these plants under study and variation of quantity determined based on the polarity of the solvent taken for extraction process, ensures its unequivocal recommendation for the use in the pharmaceutical and nutraceutical sector. Moreover, chemically, the plant contains various biologically active phytoconstituents such as flavonoids, sterols,

glycosides. Considering the results obtained in the study, we can assume that the ethanolic leaf extract of *Colocasia esculenta* Linn. might become a useful component in the treatment of bacterial and fungal diseases. The development of more purified products and the active components responsible for its antibacterial and antifungal property of *Colocasia esculenta* should be emphasized as well as the inclusion of the herbal medicines in the treatment of various infections should be encouraged. Further well designed studies are necessary to throw light of the various uses of herbal drugs for the benefit of mankind.

Acknowledgement:

Authors acknowledge the technical support of Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

References

1. O. Victor Njoku and Chidi Obi, "Phytochemical constituents of some selected medicinal plants," African Journal of Pure and Applied Chemistry, 3(11) 228-233 (2009)
2. Tchiakpe L, Balansard G, Bernard P, Dalziel JM The useful plants of west tropical Africa. Planta Med 39: 257. (1979)
3. Burkill HM (2000) .The useful plants of west tropical Africa. Royal Botanic Gardens, Kew, UK
4. Patricia RP, Joab T. Crude extract from taro (*Colocasia esculenta*) as a natural source of bioactive proteins able to stimulate haematopoietic cells in two murine models. Journal of functional foods.;18:333-343. (2015)
5. Sofowora AE (1993) . Medicinal plants and traditional medicine in Africa, 2nd (Edn), Spectrum Books Ltd., Ibadan, Nigeria 289.
6. Nakade Dhanraj B, Mahesh S. Kadam, Kiran N. Patil and Vinayak S. Mane, Phytochemical screening and antibacterial activity ofWestern Region wild leaf *C. esculenta,"* International Research Journal of Biological Sciences 2 (10) , 18-21. (2013)
7. Maheshkumar SK and Kirti SL. Determination of total flavonoids content and quantification of rutin in *Momordica tuberosa*. fruits by RP-HPLC. Asian Journal of Traditional Medicines, 7: 220-25. (2012)
8. Yang AH, Yeh KW: Molecular cloning, recombinant gene expression, and antifungal activity of cystatin from taro (*C. esculenta Kaosiung*). Planta Med 221: 493-501 (2005)
9. Sahar, A.M.Hussein, Amani, M.D . El-Mesallamy ,Salma O.K. Othman , Abd El-Mohsen M.Soliman Identification of novel polyphenolic secondary metabolites from *Pistacia Atlantica* Desf. and demonstration of their cytotoxicity and ccl_4 induced hepatotoxicity in rat.Egypt. J.Chem. 63 (12),117130, DOI: 10.21608/ EJCHEM. 2019. 16297. 1994 , (2020)
10. Amani MD El-Mesallamy, Sahar AM Hussein, Gehan NA Gad, Mohamed H Abd-Elazem, Eman M.M Ebrahem, Cytotoxicity and Anti-microbial Activity of Aqueous Methanolic Extract of *Zingiber officinale Roscoe* Zingiberacae, European Journal of Medicinal Plants Journal, 19(2);1-8,DOI:10.9734/EJ MP / 2017/33171 (2017)
11. Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 31: 53–58 (2007)
12. Sahar A M Hussein, Amani M D El-Mesallamy, Ahmed M A Souleman, Mona A Mousa. Cytotoxic Activity of Bioactive Compound from *Caesalpinia ferrea* Martius, Fabaceae. International Journal of Pharmacognosy and Phytochemical Research, ijppr 8 (12): [2080-2084], http://ijppr.com/vol 8 issue 12 (2016)
13. Kivilompolo M, Oburka V and Hyotylainen T. Comparison of GC-MS and LC-MS methods for the analysis of antioxidant phenolic acids in herbs. Analytical and Bioanalytical Chemistry, 388: 881-887. (2007)
14. McCune LM, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J Ethnopharmacol 82: 197–205 (2002)
15. Brand-Williams W., Cuvelier M.-E. and Berset C., Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28(1), 25-30 (1995)
16. Mahmoud A Nawwar, SA Hussein, AM El-Mousallami, AN Hashim, MA Mousa, MH Hetta, MA Hamed, V Werner, A Becker, B Haertel, U Lindequist, Phenolics from *Caesalpinia ferrea* Mart. : antioxidant,cytotoxic and hypolipidemic activity. Die Pharmazie 70(8), 553-558 (2015)
17. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. Antibiotic susceptibility testing by standardized single disc method. American Journal of Clinical Pathology, 44:493-496. (1966)
18. M. J. Pelczar, E. C. S. Chan and N. R. Krieg, "Microbiology Concepts and applications," McGraw-Hill Inc., New York, 967. (1993)
19. Reddy PS, John MS, Devi PV. Detection of vancomycin susceptibility among clinical isolates of MRSA by using minimum inhibitory

concentration method. Int J Res Med Sci. **3**(6): 1378–1382. (2015)
20. Amani M.D. El-Mesallamy , Nabil Abdel-Hamid , Lamis Srour , Sahar A. M. Hussein, Identification of Polyphenolic Compounds and Hepatoprotective Activity of Artichoke (*Cynara Scolymus* L.) Edible Part Extracts in Rats,. Egypt.J.Chem. 63,(6) 2273 - 2285 (2020)
21. Iwashina T, Konishi T, Takayama A, Fukada M, Ootani S. Isolation and identification of the flavonoids in the leaves of taro. Ann Tsukuba Botanical Garden 18:71-4. (1999)
22. Mohammad A and Elham KK. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. Journal of Paramedical Sciences, 4 : 119-138. (2013)