International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 22-06-2019; Accepted: 25-07-2019
www.pharmacyjournal.in
Volume 4; Issue 5; September 2019; Page No. 51-57

Morphological and anatomical property, phytochemical screening and antibacterial activity of *Colocasia Gigantea* (Blume) Hook. F

Vo Thi Thanh Phuong
CanTho University, Vietnam

Abstract
The objective of this study was to characterize the morphological and anatomical properties by double-dyeing with carmin vert d'iode, determine chromosome number of root tip by using hypo-osmotic shock method, detect qualitatively phytochemical compositions and assess the antibacteria against E. coli and B. subtilis of leaf and tuber extraction of Colocasia gigantea. The results showed morphological characters and microscope transverse sections of fresh leaf, tuber and root of C. gigantea. The chromosome number at root tip was 2n = 28. The preliminary phytochemical components were detected at leaf extract as steroids, flavonoids, alkaloids, tannins, glycosides and coumarines. At tuber, the chemical components were the same except alkaloids. Saponins was absent in both leaf and tuber extract. The antimicrobial activity against E. coli and B. subtilis was showed that leaf and tuber methanol extraction of C. gigantea inhibited the growth of both bacteria at 200 mg/ml, 400 mg/ml and 800 mg/ml) (p <0.05). At both leaf and tuber extraction, the diameter of inhibition zone against B. Subtilis was statiscally different from that of E. coli (p <0.05). C. gigantea is used as a food ingredient and traditional medicine.

Keywords: colocasia gigantea, methanol extraction, e. coli, b. subtilis, phytochemical

1. Introduction

Colocasia gigantea, a tropical Asian herb, belongs to Araceae family. The folk names of *Colocasia gigantea* are *Bạc hà* in Sonthern Vietnam and *Dọc mùng* in Northern Vietnam [18]. *C. gigantea* is an erect, stemless herb with milky sap (latex), tender perennial petioles and medium fibrous inedible corms. From its apex, the whorl of pale green, soft petioles attached with single large leaf. Leaf has heart-shaped to arrowhead-shaped, 4-6 primary pinnate venation, undulate margin and egg-shaped leaf sinus. *C. gigantea* propagates mostly by rhizomes (tuber or corm). For hundreds years ago, *C. Gigantea* has been wild or cultivated for ingredients of traditional foods as *canh chua* (sour soup), *bún* (rice vermicelli soup) or *dưa chua* (salted pickles) or for animal feeding (as pig, rabbit, fish...). Nowaday, it becomes economy botanny with hundreds of hectare crops in Vietnam. Other Southeast Asia countries, India, Thailand, Japan, Bangladesh, Pacific islands use leaves or tuberr of *C. gigantea* as vegetable or minor food [6]. For traditional medicine, the tuber of *C. gigantea* is used to reduce fever, treat drowsiness, ameliorate stomachache, combat infection, heal wounds and loosen phlegm [23]. For in vitro study, the tuber extraction from *C. Gigantea* has the anticancer activity by inhibiting the proliferation of cervical cancer Hela cells and stimulating the growth of human white blood cell [23]. The ethanol extract of *C. gigantea* induced cytotoxicity and reduced the reproductive integrity of HeLa cells. The extracts of *C. gigantea* showed antitumor activity in Dalton's lymphoma transplanted Swiss Albino mice [7]. Another research of Devi and Jagetia [6] showed the presence of various phytochemicals such as polyphenols and flavonoids in the extraction of *C. gigantea* and its potentials in the scavenge of free radicals. Phytochemicals are resposible for anti- Mutagenic, anti-carcinogenic, anti-inflammatory and anti-oxidant properties [19, 31, 34]. Many medicinal and herb plants possessing these phytochemical constituents that have high potential for developing pharmaco drugs.

The objective of this study was to explore the morphological and anatomical properties, and determine chromosome numbers of *C. gigantea*; analyse qualitatively the phytochemical constituents flavonoids, phenols, tannins, steroids, saponins, glycosides and alkaloids and assess the antibacteria against *E. coli* and *B. subtilis* of leaf and tuber extraction of *C. gigantea.*

2. Materials and Methods

2.1. Collection of Plant Material

Colocasia gigantea (Blume) Hook.F. was collected at Cantho province, Mekong Delta, VietNam. All matural plants consisting of blade, petiole, corm and root were washed to remove dust and dried under room temperature.

2.2. Moisture Content

Plant material was separated into two different parts as tuber (consisting of corm and root) and leaf (consisting of blade and petiole). Weight 5g fresh tuber/ leaf and placed the sample in a hot air oven initially at 50ºC until the dry weight of the samples become constant. According to Krishnapriya and Suganth [14], the moisture content was determined as the percentage moisture: Moisture (%) = (Fresh weight – Dry weight)/Fresh weight.

2.3. Morphological and anatomical study

Morphological study

Morphological features were evaluated by physical observation and measurement of physiognomic characteristics of fresh specimens of *Colocassia gigantea.*

Anatomical study
Tubers (corm and root) and leaves (blade and petiole) were keep intact to investigate or sliced thin. At leaf, thin slices 8 mm in length including leaf lamina and vein. At petiole, select 3 segments (the upper part near the leaf blade, the middle section and the lower section near the tuber). Split along these petiole segments (edge 5-8 mm) and cut thin slices horizontally. For tubers, peel off shaggy dry bark and shell, cut thin slices horizontally (edges 5-8 mm). At young root, cut thin slices horizontally of roots. The transverse sections of fresh leaf, tuber and root samples were cut and double-stained with carmine alum laque–iodin green dye. Carmin stains pink cellulose cell wall and green iodine stains green carpentry cell wall. A Olympus light microscope was used to view the slides. Microphotographs were obtained by using a Olympus digital camera attached on the microscope eyepiece.

Estimate chromosome number
Root tips was collected, treated hypoosmotic shock by natri citrat 0,5% for 45 minutes, fixed in Carnoy' solution (ethanol: acetic acid = 3: 1) for 4 hours and stored in 70° ethylic alcohol at 5°C for futher research. Make preparation and counted chromosome numbers of meristem of root tip at pro-metaphase plate [25]. One sample T – test was used to estimate chromosome number of *C. gigantea*. P value <0.05 was considered statistically significant different.

2.4. Qualitative analysis of the phytochemicals
Preparation for plant extract
The plant material was seperated into leaf and tuber. Each part cut into small pieces and dried in hot air oven at 55^{0}C until the dry weight of the samples become constant. Grind samples to a fine powder, which was used for the chemical analysis.

Methanol extraction
The 50 g each sample powder (leaf or tuber) was extracted with 500 ml methanol using Soxhlet apparatus at 60°C for 8h. Sample extraction was evaporated to dryness under reduced pressure by using rotary evaporator [27]. The sediment powder was extracted with solvent. The finally combined sample extracts were used for further studies.

Test for steroids
Salkowski test (Conc. H_2SO_4): To 2ml extract, add 1 ml chloroform. Each drop of Conc. H_2SO_4 from the sides of the test tube. To stablize for moments. Red/ orange color appears at the lower layer indicates the presence of steroids [5, 19].
Libermann-Buchard test (Acetic anhydrit $(CH_3CO)_2O$ (20 ml), conc. H_2SO_4 (1ml)): To 2 ml extract, add 2 ml chloroform, 1-2 ml acetic anhydride and 2 drops of concentration H_2SO_4 from the side of test tube. Observe a brown/ green ring at the junction of two layers shows the presence of steroids [5].

Test for flavonoids
Shinoda test: To the 2 ml of extract, 2 ml methanol, 5 drops HCl was added. Followed by adding few fragments of magnesium ribbon. Pink, red or occasionally green to blue colour appeared to indicate the presence of flavonoid [5, 10].
Ammonia test: To the 1 ml of extract, add few drops of 10% Conc. H_2SO_4 and 1ml of ammonia. The formation of greenish yellow precipitate indicated the presence of flavonoids [14, 19].

Test for alkaloids
Dragendroff test: To 2 ml extract solution, add few drops of Dragendroff's reagent (excess of KI + $BiNO_3$ solutions), reddish brown precipitate is produced [10].
Wagner test: To 2ml extract solution, add few drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100 ml of water), reddish brown precipitate is indicated the present of alkaloid [10].

Test for glycosides
Fehling's test: Added 1ml of water and few drops of equal amount of both Fehling A and B's solution in 1 ml extract and heat in wath bath. The appearance of brick red precipitate indicates the presence of glycosides [5].
Keller-Kiliani Test: To 2 ml of extract, add 1 ml of glacial acetic acid, few drops of 5 % $FeCl_3$ and Conc. H_2SO_4 were added. A reddish brown ring forms at interface and upper acid acetic layer turned bluish green that visualise the presence of glycosides [19].

Test for tannins: By using ferric chloride test, to 2 ml extract, add 3 drops of $FeCl_3$. Blue, green or brownish green color indicate the presence of tannins [5, 10, 19].

Tests for saponins
To 2 ml of extract add 20 ml of water and shaken. Stable for 15 minutes. Persistent foam indicated the presence of saponins [10, 19].

Test for coumarins
By rsing the reaction of opening and closing lacton, add into each test tube 2 ml extract. Add 0.5 ml of 10% NaOH solution in one test tube 1 and heating. The test tube 2 was used as control. After cooling, add 4ml H_2O in each test tube. Each drops of HCl in tube 1. Cloudy or precipitate in tube 1 shows the presence of coumarins.

2.5. Antibacterial screening
Methanol extraction
The 30 g samples powder (leaf and tuber) were extracted with 450 ml methanol using Soxhlet apparatus at 60°C for 8 h. Sample extraction was evaporated to dryness under reduced pressure by using rotary evaporator [27]. The sediment powder was extracted consicutively with solvent. The finally combined sample extracts were used for further studies.

Test organisms
Escherichia coli and *Bacilus subtilis* were provided from Institude of Research and Development of Biotechnology, CanTho University, Vietnam. *E. coli* and *B. subtilis* were cultured in LB agar at 37ºC in 24 hours. Adjust the turbidity of suspension (compared to 0.5 McFarland turbidity standards) that was equivalent to 10^6cfu/ml.

Antimicrobial assay
The agar well diffusion method was used to investigate the antibacterial activity of *C. giagentea* extraction at concentration of 200mg/ml, 400mg/ml and 800mg/ml. 100μl each bacterial suspension was spread uniformly on each Petri dish, containing solidity LB Agar. After 24h

inoculated, 5 of 6mm diameter wells were cut on the agar of plate. In every plate, 100µl of 3 different concentration of leaf or tuber extracts dissolved in DMSO, 10µl tetracycline was prepared as 30mcg/ml (w/v) concentration in sterile distilled water and control DMSO well added. All plates were incubated at 37^0C for 24hrs. Diameter of the inhibition zone were measured in mm. The experiments were repeated 3 times. Statistical analysis of the data was done by using One-way ANOVA with posthoc test and p value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Morphological characteristics

Morphologically, *C. gigantea* is an erect, stemless with milky sap (latex), tender perennial petioles and medium fibrous inedible corms. The plant grows well in wet or marshy soil under full sun from its corms. From one tree, it is propagaged into bush with many new ones around. Tuber is inedible that grows underground from bulging root, also called corms.

Tuber is grey/brown colour and slightly round – shaped. From its apex, the whorl of pale green, soft petioles attached with single large leaf are grown. The lower part of tuber is rhizomes of different shapes and sizes. Each tuber has a lot of burning with the skin is often corky, rough and sloughing. Each bush can have many tubers which is high in fiber and starch inside. Tubers contain many toxins (calcium oxalate) so they cannot be eaten.

Leaf consists of petiole and blade leaf. Petiole is developed from rhizomes in the ground which clump together to form a fat, fake body and growing straight on the ground. Petiole is greenish, covering with milky sap (latex) and staggered on tubers. The length of leaf sheath ranges from 0.5 to 1.2 m. Petiole is oblong, the closer the leaf blade is, the more slender the leaf sheath. A lower half of petiole has a deep concave that extend about 2-4 cm to embrace the other petioles to form fake stem. Two sides of concave are two thin edges that are close to each other and gradually disappear at middle of petiole. The upper half of the petiole is cylindrical round - shaped. Petiole is thick, spongy and only edible part of *C. gigantean.* Blade leaf has single, wide plate, heart-shaped to arrowhead-shaped, 4-6 primary pinnate venation, undulate margin, egg-shaped leaf sinus and hearted shape lobeds. Leaf tips with tail. The upper face of leaf is pale, polished green and the underneath is lighter. The mature leaves is 20-50 cm in length and 20–40 cm in width. The main venation has a "Y" shape that runs along the length of the blade leaf and reaches 2 sides of lobes. Along the main venation are 5 to 6 pairs of sub-venations. The upper surface of the venation is less convex than the lower surface. The leaf blade attaches to the petiole at the intersection of the Y-ribs on the leaf blade.

3.2. Anatomical characteristics

Root transverse section

The outermost layer is epiblema. Some epiblema cells prolong to form the typically unicellular root hairs. In older root, there is a trace of root hair. Exodermis is 2-3 layers of suberisation cells to form a zone for protecting the internal tissues. Many layers of soft parenchyma cells are located inside with diffferient shape and size. Some transition sections of root show small to large inter-space between soft parenchyma cells. Epidermis is uniseriate, containning a row of tabular cells which are attached end on end. Uni-seriate pericycle are strucured by thin-walled parenchyma cells, located next to endodermis. Vascular tissue is 12-13 bundles of xylems and phloems arranged in circle inside of epidermis. The phloem bundles are arranged in circle or oval clusters. There are 6-8 round or oval xylem bundles with different shape and size located next to phloems. Proto- xylem occurs on the outer side and meta-xylem the inner. Some small conjunctive parenchyma cells are present between xylem and phloem patches. The central part of the stele is a fairly large pith. Druse and raphide crystals are scatered in root (Figure 1).

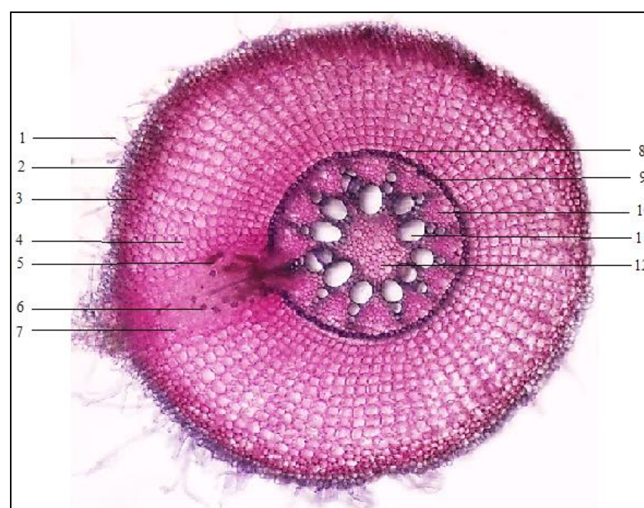

Fig 1: Root in transverse section

1. Root hair, 2. Epiblema, 3. Suberin, 4. Parenchyma, 5. Raphide crystal, 6. Druse crystal, 7. Lateral root, 8. Epidermis, 9. Pericycle, 10. Phloem, 11. Xylem, 12. Pith

Tuber transverse section

Flesh of tuber is structured by polygon parenchyma cells. The calcium oxalate crystals are encountered in forms of druses and raphides (both single and bundle) which is densely distributed in tubers. Fibers are occurred also (Figure 2).

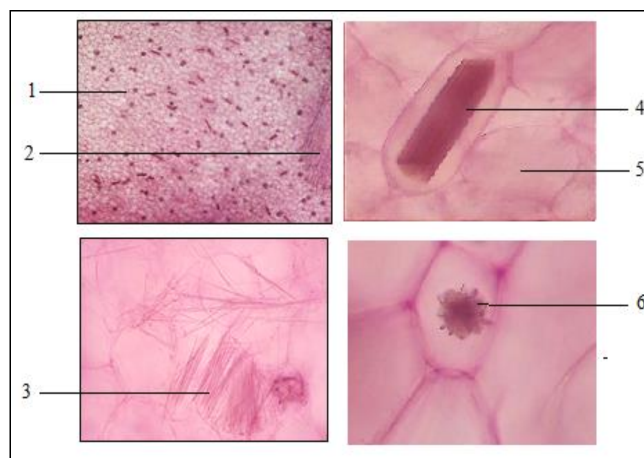

Fig 2: Transverse of tuber flesh

1. Tuber flesh with crysstals, 2. Fibers, 3. Single raphide crystals, 3. Raphide crystal, 5. Paranchyla cell, 6. Druse crystal

Lamina transverse section

Leaf blade

The almost outside of the leaf blade is a epidermis layer with various size, impregnated – cuticle cells. Under epidermis is 2-3 layers of palisade mesophyll cells which are lightly oblong rectangle, various size and wall-cellulose structure. The spongy mesophyll cells are polygon, round or oval shape, differient size and close together with tiny intercellular spaces. The lower epidermal cells are laced with thick and irregular cutin. Druses are distributed on the upper surface of the leaf blade more than the lower part. Druse, styloid and raphide crystals are scattered in parenchyma (Figure 3).

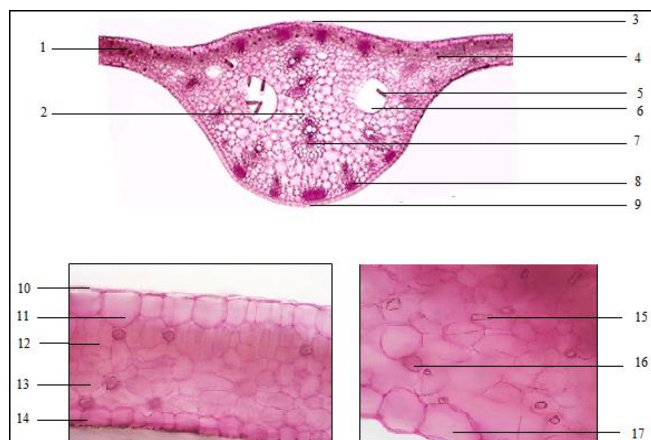

Fig 3: Lamina transverse section

1. Blade, 2. Vein, 3. Upper epidermis, 4. Druse crystal, 5. Raphite crystal, 6. Air cavity,
7. Xylem and phloem, 8. Secretory tube, 9. Lower epidermis of vein, 10. Cutin, 11. Upper epidermis of blade, 12. Palisade mesophyll, 13. Spongy mesophyll, 14. Lower epidermis of blade, 15. Styloid crystal, 16. Druse crystal, 17. Lower epidermis of blade

Vein

The upper surface of the vein is less convex than the lower surface of the vein. The epidermis is a layer of various size, cutin-laced cells. Below the epidermis is 3 to 4 layers of soft parenchyma cells with small size, near-round shape and close together. The central region of the veination is the layers of parenchyma with polygon-shaped, large size cells. Between cells are tiny air spaces. Vascular bundles are scatered in parenchyma. Druse crystals are distributed more on the upper side of vein than the underside. Single raphide The single is scattered throughout soft parenchyma.

Petiole transverse sections

Epidermis contains cells laced cutiles. Beneath the epidermis or hypodermis is a continuous bands of angular, small size cells of collenchyma. Alternating is loop of the secretory tube containing secretions. The grounded tissue is the layers of parenchyma cells with varous size and polygonal shape. A numerous small to medium-sized air cavities that are embedded calcium oxalate crystals. Vascular bundles are xylems oriented with phloems that face perimeter. The calcium oxalate crystals that are represented by druses and single/bundle raphide are distributed with high density from hypodermis to central of unpetiole (Figure 4).

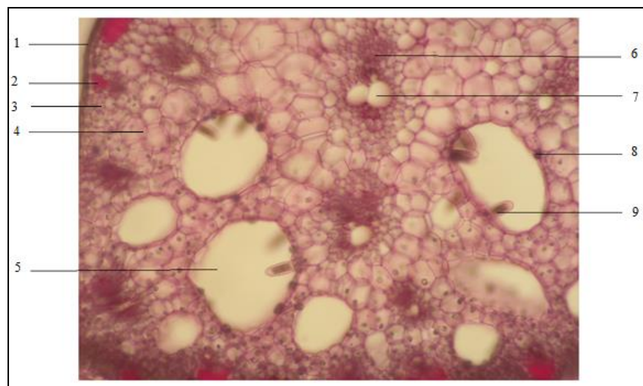

Fig 4: Petiole transverse sections

1. Epidermis,, 2. Secretory tube, 3. Collenchyma, 4. Parenchyma, 5. Air space, 6. Xylem,
7. Phloem, 8. Druse crystal, 9. Raphide crystal

3.3. Chromosome estimatimation

Yang *et al.* [33] found chromosome number of *C. gigantea* was 2n = 28. Using method hypo-osmosic shock, the number of chromosome of *C. gigantea* was estimated 2n = 28,2 ± 0,76 (p > 0.05) (Figure 5).

Fig 5: Chromosome number

3.4. Water content and yield extract of C. gigantea

Water content of *C. gigantea* was 88,46 ± 0,91 % at tuber and 51,66 ± 0,58 % at leaf. The extract yield of *C. gigantea* was 13,27 % at tuber and 26.43 % at leaf. For *Colocasia esculenta*, its moisture content were 73,1% at tuber and 82,7% at leaf (Gopalan *et al.,* 1989). Water content of *Colocasia esculenta* tuber was 56.8% - 57% [14, 19].

3.5. Qualitative phytochemical screening

Phytochemicals that were detected at leaf methanol extract of *C. gigantea* were steroids, flavonoids, tannins, glycosides, alkaloids and coumarines. For tuber methanol extract, the phytochemical constituents were steroids, flavonoids, tannins, glycosides and coumarines. There was no alkaloids found in tuber extract and no saponins to be revealed in both leaf and tuber extract. The results showed on table 1.

Table 1: Qualitative analysis of phytochemicals in leaf and tuber extract of C. Gigantea

Phytochemicals	Test	Leaf	Tuber
Steroid	Salkowsky	+	-
	Libermann-Burchard	+	+

Flavonoid	Shinoda	+	+
	Ammonia	+	+
Alkaloid	Dragendorff	-	-
	Wanger	+	-
Glycosides	Fehling	+	+
	Keller-Kiliani	+	+
Tannins	Ferric Chloride	+	+
Saponins	Foam	-	-
Coumarine	Open and close Lacton	+	+

Raju [24] found that the petroleum ether extract of *C. gigantea* petiole in Kerala (India) possessed the phytochemicals such as carbohydrates, protein, amino acid, alchohol, esins, glycosides, tannins, diterpenes and phenols, but negative in starch, saponins and flavonoids. Devi & Jagetia [7] found that the rhizomes (tuber) extraction of *C. gigantea* indicated the present of various phenols and flavonoids. At genus *Colocasia*, Krishnapriya and Suganthi [14] proved that methanol tuber extract of *C. esculenta* presents alkaloids, glycosides, flavonoids, terpenes, saponins and phenols. Another phytochemical analysis of tuber of *C. esculenta* revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins and phenols [19]. Study on leaf of *C. esculenta*, Keshav *et al.* [13] identified the presence of phytochemicals in ethanol, methanol and chloroform extract such as alkaloids, glycosides, flavonoids, terpenoids, saponins, oxalates and phenols. For phytochemical quality test on the extract of *C. affinis* leaf, Mondal *et al.* [17] affirmed the presence of alkaloids, flavonoids and tannins. The results of this study demonstrated the presence of second metabolites, phytochemicals, in the extract of *C. gigantea* the same as the other plant in genus *Colocasia*.

3.6. Antibacteria activity of Methanol extraction of C. gigantea again E. coli và B. subtilis

In this study, the agar well diffusion method was used to assess the antibacterial activity by measuring the inhibition zone against the test microorganisms. The results of zone inhibition diameter of *C. gigantea* extraction again *E. coli* and *B. subtilis* show on table 2. DMSO that used as negative control showed no inhibition zone found around these wells. The inhibition zone of standard well were measured from 20-24 mm. The leaf and tuber methanol extraction of *C. gigantea* inhibited the growth of both gram negative and gram-positive bacteria. The diameter of inhibition zone against *E. coli* and *B. subtilis* of both tuber and leaf extraction to be statistically different at 200 mg/ml, 400 mg/ml and 800 mg/ml) ($p < 0.05$). At both leaf and tuber extraction, the diameter of inhibition zone against *B. Subtilis* was statiscally different from that of *E. coli*.

Table 2: Diameter of inhibition zones against E. coli and B. Subtilis

	Bacteria	Tetracycline	DMSO	200 µg/ml	400 µg/ml	800 µg/ml
Tuber	*B. subtilis*	+	-	9,66 ±0,58^{ad}	13,66±0,58^{bd}	18±1^{cd}
	E. coli	+	-	7,66±0,58^{a}	10,66±0,58^{b}	14,33±1,2^{c}
Leaf	*B. subtilis*	+	-	8,66±0,58^{ae}	14,66±0,58^{be}	19±0^{ce}
	E. coli	+	-	7,33±0,58^{a}	11,66±1,2^{b}	16,0±1,0^{c}

Values are expressed as mean ± SD and analyzed by ANOVA (p <0.05).
a, b, c: p < 0,05 when compared between different concentrations against E. coli or B. subtilis;
d: p <0,05 when compared to E. coli for tuber extract; e: p<0,05 when compared to E. coli for leaf extract.

The present study proved the ability of antibacteria of leaf and tuber extract against *E. coli* and *B. subtilis.* Raju [24] indicated that the petiole extract of *C. gigantea* showed the antibacterial activity against *E. coli* and *Staphylococcus areus.* There are not many published documents about the antibial activity of *C. gigantea.* However, many significant announcements about antibacterial activity on genus *Colocasia*, especcially *Colocasia esculenta*. Leaf aqueous extraction of *Colocasia esculenta* (Taro) collected from Hymalaya region was showed the antibacterial activity against *B. subtilis* with inhibition zone 7.03 mm and *E. coli* with inhibition zone 9.00 mm at 400mg/ml [9]. Leaf ethanol extraction of *Colocasia esculenta* showed antibacteria activity against *Staphylococcus aureus, Klebsiella sp, Escherichia coli, and Pseudomonas aeruginosa*. For *E. coli*, the inhibition zone diameter at concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ ml was alternatedly 4.6 mm, 9.2 mm, 16.2 mm and 17.1 mm. Kubde *et al*. (2010) found leaf methanol extraction of *C. esculenta* possessed the ability of antibacteria against *S. aureus, Bacillus subtilis, E. coli,* and *Proteus vulgaris.* Mondal *et al.* [17] affirmed that leaf extract of *Colocasia affinis* showed the antibacteria activity against B. *subtilis, B. cerous*, *E. coli, Salmonella* Typhi, *Speudomonas* spp.

It is evident that mechanism of antibacterial action may be due to the presence of phytochemicals such as steroids, flavonoids, tannins, glycosides, alkaloids and coumarines that cause the inhibition of biological process and finally cause dead of bacteria. Plant steroids have been reported to have antibacterial properties by causing leakages of membrane lipid of bacteria [11]. Flavonoids are antibacterial agents due the inhibition of biological process as nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, biofilm formation and membrane permeability [32]. Tannins is be toxic to bacteria because of binding to cell walls of bacteria and preventing growth and protease activity [4] interfering with the protein synthesis [26] inhibiting the biofilm formation and destroying cell wall of bacteria [8]. Alkaloids are structurally diverse compounds showed the antibacteria activity by affecting on cell division, respiratory process and membrane structure and virulence genes affection [21]. Arabski *et al.,* [2] proved that saponins do not inhibites but enhances the six *E. coli* strains growth. However, saponin in the extract of *Phyllanthus niruri* showed antibacterial activity against *Staphylococcus aureus* by involving the physiological mechanism on

membrane [1]. Coumarin has antibiotic resistance through a mechanism that involving the inhibition of bacterial efflux pumps [3] and the reduction of bacterial biofilm formation [16]. Tagousop *et al*. [29] explained the mechanism for antibacterial action of glycoside that involves in the lysis, leakage and osmotic stress conditions of cell membrane. This study showed that leaf and tuber extract of *C. Gigantea* possessed compounds with antimicrobial properties that caused the inhibition of biological process of bacteria.

E. coli is virulent bacteria strains that causes a number of diseases such as gastritis, urinary tract infection, genital infection, liver and bile infection, bronchitis, pneumonia [26]. *Bacilus subtilis* known as Gram-positive microorganisms and widespread in food, soil, water and air. It is also a stable microorganism flora of intestine gut of animal and human. The beneficial utilization of *B. subtilis* are probiotic supplement, health-status improvement, immune system stimulation and antibacteria agents [21]. However, *B. subtilis* was found the cause of an outbreak of food poisioning [22, 30] and foodborn illness [15]. *Bacillus subtilis* plays an important role in the use of probiotics, prevention of intestine inflammation, antibacterial agents, antidiarrheal effects and intestinal flora stable [28].

The high antimicrobial ability of leaf and tuber methanol extract of *C. gigantea* shows that it is possible to use as a medical source of raw materials to produce drugs, improve the treatment of infections caused by *E. coli* and *B. subtilis*. In folk and traditional medicine, *C. gigantea* is valuable for use in remedies for curing infection, epilepsy, phlegm and tuberculosis and healing wounds. Reseachers proved *C. gigantea* has antitumor and anticancer activity [7]. It is in need of further study to improve its pharmaceutical and bioactive property.

4. Conclution

Colocasia gegantea is utilized for medicinal and nutritional resource. The present study showed anatomical and morphological characteristics of root, leaf and tuber of *C. gigantea.* Phytochemical screenning results showed the presence of steroids, flavonoids, tannins, glycosides, coumarines in both methanol leaf and tuber extract. Alkaloids were only detected in leaf. Saponins was absent in both leaf and tuber extract. Leaf and tuber extract possessed antibacterial activity against *E. coli* and *B. subtilis*. Besides, *B. subtilis* showed more sensitive than *E. coli* at both leaf and tuber extract. More study about quantitatively phytochemical components and other biological activities are important to prove medicinal property of *C. gigantea.*

5. References

1. Ajibade VA, Oluwasusi VO, Ibiyemi MF, Ajenifuja1 OA, Famurewa O. Antibacterial Activity of Saponin Extracted from Phyllanthus niruri on Methicillin-Resistant *Staphylococcus aureus* (MRSA). Journal of Complementary and Alternative Medical Research. 2019; 7(1):1-9.
2. Arabski M, Węgierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of Saponins against Clinical *E. coli* Strains and Eukaryotic Cell Line. Journal of Biomedicine and Biotechnology, 2012. (http://dx.doi.org/10.1155/2012/286216)
3. Araújo R, de SA, Barbosa-Filho JM, Scotti MT, Scotti L, D RM. Cruz da, Falcão-Silva SV, de Siqueira-Júnior JP, Mendonça-Junior FJB. Modulation of Drug Resistance in Staphylococcus aureus with Coumarin Derivatives. Scientifica, 2016. (http://dx.doi.org/10.1155/2016/6894758)
4. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12(4):564-582
5. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). International Journal on Pharmaceutical and Biomedical Research (IJPBR). 2010; 1(5):150-157
6. Devi NB, Jagetia GC. Free radical scavenging and antioxidant potential of different extracts of *Colocasia gigantea* (Blume) Hook. F. in vitro. Int. Res. J Pharm. 2017; 8(10):72-81
7. Devi NB, Jagetia GC. Antitumor activity of different extracts of *Colocasia gigantea* in Dalton's lymphoma transplanted Swiss Albino mice. International Research Journal of Pharmaceutical and Biosciences. 2017; 4(4):52-82
8. Dong G, Liu H, Zhang X, Lu H, Zhou T, Cao J. Antimicrobial and anti-biofilm activity of tannic acid against *Staphylococcus aureus*. Nat Prod Res. 2018; 32(18):2225-2228.
9. Dutta S, Aich B. A study of antibacterial and antifungal activity of the leaves of Colocasia esculenta Linn. Int J Pharm Sci Res. 2018; 8(3):1184-87.
10. Dwivedi P, Dwivedi J, Dhara P, Desai S. Phytochemical analysis and assessment of in vitro urolithiatic activity of Colocasia leaves. Journal of Medicinal Plants Studies. 2016; 43(46):43-47.
11. Gauniyal PUV, Teotia S. Antimicrobial activity and phytochemical analysis of ethanolic extracts of twelve medicinal plants against oral micro organisms. Int. J Pharm. *Med. Res*. 2014; 2(1):21-27.
12. Gopalan C, Shastri BVR, Balasubramaniam SC. Nutritive Value of Indian Foods. National Institute of Nutrition. Indian Council of Medical Research, 1989, pp49. http://www.eeb.cornell.edu/biogeo/nanc/Food_Feed
13. Keshav A, Sharma A, Mazumdar B. Phytochemical Analysis and Antioxidant Activity of Colocasia esculenta (L.) Leaves. International Journal of Chemical and Molecular Engineering. 2019; 13(1):20-23.
14. Krishnapriya TV, Suganth A. Biochemical and phytochemical analysis of *Colocasia esculenta* (L.) Schott tubers. International Journal of Research in Pharmacy and Pharmaceutical Sciences. 2017; 2(3):21-25.
15. Logan, N, Bacillus A. relatives in foodborne illness. Journal of Applied Microbiology. 2011; 112:417-429.
16. Lee Jm, Kim Y, Cho HS, Ryu SY, Cho MH, Lee J. Coumarins reduce biofilm formation and the virulence of Escherichia coli O157:H7. International Journal of Phytotherapy and Phytopharmacology, 2014. DOL 10.1016/j.phymed.2014.04.008.
17. Mondal M, Hossain MS, DasAbul N, Khalipha BR, Sarkar AP, Islam MT. *et al*, Kundu. Phytochemical screening and evaluation of pharmacological activity of leaf Methanolic extract of *Colocasia affinis* Schott. Clinical Phytoscience. 2019; 5(8). https://doi.org/10.1186/s40816-019-0100-8
18. Nguyen Van Du, Bui Hong Quang, Nguyen Thi Van Anh, Tran Van Tien, Masuno T, Peter J. Matthews.

Useful aroids and their prospects in Vietnam. Aroideana. 38E (1):130-142.
19. Nayak L, Mishra D, Rath. Phytochemical Screening of Colocasia esculanta. J Pharm Adv Res. 2019; 1(3):176-180.
20. Olmos J, Paniagua-Michel J. subtilis Bacillus. A Potential Probiotic Bacterium to Formulate Functional Feeds for Aquaculture. J Microb Biochem Technol. 2014; 6(7):361-365
21. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Frontiers in Microbiology. 2019; 10:1-28.
22. Pavić SM, Brett I, Petrić D, Laštre M, Smoljanović M, Atkinson A. *et al*. An Outbreak of Food Poisoning in a Kindergarten caused by Milk Powder containing toxigenic *Bacillus subtilis* and *Bacillus licheniformis*. Archiv fur Lebensmittelhygiene. 2005; 56:1-24.
23. Pornprasertpol A, Sereemaspun A, Sooklert Chutimon K, Satirapipatkul K, Sukrong S. Anticancer Activity of Selected *Colocasia gigantia* Fractions. J Med Assoc Thai. 2015; 98(1):98-106.
24. Raju RS. Characterization of bioactive components from petiole of two varieties of Colocasia seen in Kerala. International Journal of Advance Research, Ideas and Innovations in Technology. 2016; 3(2):1382-1386.
25. Sharma AK, Sharma A. Chromosome technique-Theory and Practice. Third Edition. Butterworth-Heinemann, 1980.
26. Shihabudeen MS, Priscilla H, Thirumurugan K. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma Sciences and Research. 2010; 1(10):430-434.
27. Subhash C, Sarla S, Jaybardhan S. Phytochemical screening of garhwal Himallaya wild edible tuber *Colocasia esculenta*. International Research Journal Pharmacy. 2012; 3(3):181-186.
28. Suva MA, Sureja VP, Kheni DB. Novel insight on probiotic Bacillus subtilis: Mechanism of action and clinical applications. Journal of Current Research in Scientific Medicine. 2017; 2:65-72.
29. Tagousop CN, Tamokou JD, Ekom SE, Ngnokam D, Voutquenne-Nazabadioko L. Antimicrobial activities of flavonoid glycosides from Graptophyllum grandulosum and their mechanism of antibacterial action. BMC Complement Alternative Medicine. 2018; 18(1):252-262.
30. Ueda S, Kuwabara Y. Comparison of *Bacillus subtilis* Strains from a Food Poisoning Outbreak and Other Sources Analyzed by Pulsed-Field Gel Electrophoresis. Biocontrol Science. 1999, 4(1):51-54.
31. Umaru IJ, Fasihuddin BA, Otitoju OO, Hauwa AU. Phytochemical Evaluation and Antioxidant Properties of Three Medicinal Plants Extracts. Medicinal & Analytical Chemistry International Journal 2018, 2(2). (https://medwinpublishers.com/MACIJ/MACIJ16000119).
32. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Current Medicinal Chemistry. 2015; 22:132-149.
33. Yang J, Yi T, Li H, Gong X. A Cytological Study on three Species of Colocasia (Araceae) from Yunnan. Caryologia. 2003; 56(3):323-327.
34. Zhai T, Li S, Hu W, Li D, Leng S. Potential Micronutrients and Phytochemicals against the Pathogenesis of Chronic Obstructive Pulmonary Disease and Lung Cancer. Nutrients. 2018; 10(7):8-13.