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Preliminary Pharmaceutical Active Ingredient and Micronutrient Evaluation of the Leaf of *Corchorus olitorius* (Ahiahara)

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Abstract

Pharmaceutically active ingredients and micronutrient evaluation of the leaf of *Corchorus olitorius* were determined. The phytochemical screening using some standard methods indicated that alkaloids, flavonoids, saponins, terpenoids, phenols and tannins were present. The quantitative analysis showed the value of flavonoid as (6.25±0.5)%, Alkaloid (6.3±0.3)%, Saponin (8.01±0.6)%, Phenol (1.16±0.01)% and Tannin (1.03±0.2)%. The HI8300 multiparameter photometer result of the mineral element analysis showed that the leaf has nine out of the ten minerals analyzed for in various concentrations, the result showed that one kg of the leaf contain 0.072 mg of chromium, 15 mg of iron, 4 mg of manganese, 2.4 mg of iodine, 16 mg of phosphorus, 0.1 mg of calcium, 0.88 mg of zinc, 160 mg and 150 mg of magnesium and potassium respectively, while copper was absent. The leaf extract was subjected to different chromatographic techniques which include thin layer and column chromatograph for separation and purification, Antimicrobial screening using disc diffusion method revealed that the column eluates were active against some tested microorganism with the following zone of inhibition, Pseudomonas aeruginosa (7-15 mm), *Eschericia coli*, (3-18 mm), *Shigella species* (12-17 mm) *Staphylococcus aureus* (1-8 mm), *Aspergillus nomius* (2-4 mm) and *Aspergillus flavus* (4 mm). The result portrayed that the eluates were highly bactericidal and fungicidal and of broad spectrum activities. The antimicrobial activities were compared with known standard antibiotic like ciprofloxacin and ketoconazole and was discovered that the eluates were potent and effective in both fungi and bacteria close to the antibiotics. The results scientifically validate the efficacy of the plant as acclaimed ethonomedically.

Keywords: Pharmaceutical; Phytochemicals; Micronutrients; Antimicrobial; Column eluates; *Corchorus olitorius*

Introduction

Medicinal plants are frequently used as raw materials for extraction of active ingredients which are used in the synthesis of different drugs, like in case of laxatives, blood thinners, antibiotics and antimalaria medications, which contain active ingredients from plants called phytochemicals [1].

Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic or acute diseases. The presence of these bioactive components are said to confer the user with resistance against bacterial, fungal and pesticidal pathogens. These bioactive components are said to be responsible for the antimicrobial effects of plant extracts [2]. *Corchorus olitorius* is an edible leafy vegetable, of the family *Malvaceae*. *Corchorus olitorius* is widely found in tropical and subtropical areas from Asia to Africa valued as food and for its strong fiber. It has long been used as food staple since ancient times by Jewish people and

Egyptians hence derived its English names Jew's mallow and Egyptian spinach. *Corchorus olitorius* leaves have been reported to be very nutritious, rich in calcium, iron, protein, vitamin A, C and E, thiamin, riboflavin, niacin, folate, and dietary fibers. *Corchorus olitorius* is usually cooked as stew, forming thick slimy syrup similar in consistency to okra usually taken with rice or other starchy staple [3]. *Corchorus olitorius* has been claimed to be a medicinal plant that is widely used in different localities for treatment of diseases. The seeds have been reported to be used as a purgative and leaves as demulscent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria. On preliminary analysis, seeds have been found to contain cardenolide glycosides [4]. *Corchorus olitorius* leaf has been reportedly used in folklore for managing diabetes and hypertension [5]. Prevent internal bleeding, protects eye health, reduces restless leg syndrome, supports skin health and cell growth, fights off colds and flu, reduces cholesterol, prevent cancer, maintains healthy teeth and gums, prevents asthma, protects healthy hair and skin [6].

Other traditional acclaimed uses and benefits of *Corchorus olitorius* includes traditional remedy for aches and pains, fever, dysentery, enteritis, pectoral pains, tumors, ascites, piles, tumor to restore appetite and strength. Treatment of liver disorders, dyspepsia and gonorrhea [6]. It has been reportedly found also to possess gastroprotective effect, anti-tumour, anti-diabetes, anti-inflammatory and antipyretic [3]. Hence the research aimed at identifying and evaluating the bioactive ingredient that can be used in pharmaceutical and to determine the micro-nutrients in the leaf.

Material and Methods

Plant Identification and Collection

Fresh leaf of *Corchorus olitorius* was collected from a local farm in Ohii in Owerri-West Local Government of Imo State Nigeria. The plant material was identified by Prof F.N Mbagwu of Plant Science and Biotechnology Department of Imo State University, Owerri as *Corchorus olitorius* from the family of *Malvaceae.*

Preparation of the Sample for Analysis

Fresh *Corchorus olitorius* leaf samples were washed with water to remove sand and dirt, and then dried in a room at ambient temperature of about 33^{0}C for 2 weeks. The plant material after drying was powdered with new Corona mechanical grinder 2013 model. The powdered leaf sample which weighed (2.2kg) was stored in amber coloured Winchester bottle for analysis.

Ethanol Extract

The powdered *Corchorus olitorius* 500g was percolated and shaked with 1000ml of redistilled ethanol (99%) for 24 hours. The extract was filtered and concentrated with rotary evaporator at 55^{0}C.

Qualitative and Quantitative Phytochemical Analysis

Qualitative and quantitative phytochemical analysis of the plant extracts were carried out in order to confirm the presence and quantity of phytochemicals presence in the leaf using some standard methods by [7-9]. The results were recorded in appropriate tables.

Separation and Purification (Chromatographic Technique)

Column chromatography was done using silica gel (Merck 60-200 mesh, 400 g). Column chromatography silica gel was washed several times with petroleum ether and chloroform to remove oily materials. Thin layer chromatography (TLC) was carried out on 20 x 5 glass plate with TLC silica gel. Silica plates were made by coating them with liquid slurry of silica gel and left to dry for 24 hours and activated in an oven temperature before using. Iodine crystals were used to develop the spots.

Thin Layer Chromatography (TLC)

Procedure: 50 g of Silica gel were mixed with 100 cm^3 of distilled water i.e. (1:2) and was stirred thoroughly until homogenous slurry was obtained. Chromatographic plates were cleansed with acetone to eliminate any grease or moisture and were coated with slurry and allowed to dry for about 24 hours. The TLC plates were activated by heating in an oven at less than100^{0}C for 30 mins. The extract was spotted on the plates with distance marked 2 cm above the edge of the plate. The spotted plates were subsequently developed in the most suitable solvent system of petroleum ether, chloroform and methanol. After the solvent must have traveled the distance, the plates were brought out and allowed to dry. Each plate was placed in an iodine tank for identification, record of the active spots were taken and the retardation factor (RF values) of the individual spots or components were calculated using the formula.

$$R_f = \frac{\text{Movement of sample}}{\text{Movement of Solvent front}}$$

Column Chromatography

This was used to purify the compounds. The 25 g of silica

gel was used to prepare slurry for the chromatography.

Preparation and packing of Column: A glass column of length 100 cm and diameter 2.5 cm was packed with silica gel 60-200 mesh using slurry method. 2 g of the ethanol extract was redissolved in 20 mL of petroleum ether and 20 g of silica gel was added and mixed thoroughly with the extract. It was allowed to dry to a free flowing powder. The body of the column was continuously tapped with the hand to remove any trace of air bubbles. The packed column was washed twice with 100 mls of petroleum ether. After packing and regulating of the flow of the column to about one drop in 15 seconds, the extract mixed with the silica gel was added, and covered with dry silica gel to prevent direct pouring of the solvent on the separating sample.

The column chromatograph separation was done with varying concentrations of petroleum ether and chloroform in the ratio of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100 then chloroform and methanol in the ratio of 90:10, 80:20 and 70:30. The eluting solvent was collected at 50 mL volumes and each allowed to evaporated to about 5 mL volume at room temperature to concentrate any eluted compound. Each concentrate was then spotted on TLC plates. After developing the plates, eluates that gave a single spot were used for the antimicrobial study.

Determination of Antimicrobial Activity of Extracts

Source of Test Organisms: Pure cultures of five test bacterial organisms; *Staphylococcus aureus, Streptococcus species, Escherichia coli, Pseudomonas aeruginosa, and Shigella species* and four test fungal organisms; *Aspergillus tamari, Aspergillus niger, Aspergillus flavus and Aspergillus nomius* species used in this study were obtained from the Department of Microbiology, Imo State University, Owerri.

Antimicrobial Activity: The antimicrobial activity of the extracts was determined using modified agar well diffusion technique. [10] Muller Hinton and Sabouraud dextrose agar plates were each inoculated with each bacterial and fungal strain respectively. The inoculated plates were allowed to set and then dry. A sterile cork borer of 6mm diameter was used to bore uniform wells on the surface of the agar. Exactly 0.1ml of each of the extracts were placed in the wells. Ciprofloxacin (25mg/ml) and Ketoconazole (20mg/ml) were used as positive control for bacterial and fungal strains respectively. The plates were incubated at 37^0C and allowed to stay at room temperature for 24 hours for bacterial strains and 72 hours for fungal strains. The results of antimicrobial activities of each eluate was measured as the inhibition zone diameter in millimeters and recorded in appropriate tables

Micro-nutrient determination

Digestion of sample: 5 ml of 65 % HNO_3 was added to 0.5 g of the sample in a beaker, the mixture was boiled gently for 30–45 min. After cooling, 2.5 ml of 70 % $HClO_4$ was added, and gently boiled until dense white fumes appeared. The mixture was allowed to cool, 10 ml of deionized water was added followed by further boiling [11].

Micro nutrients Analysis: All elements were determined using Hanna 8300 multiple parameter photometer to know the concentrations of the trace metals present in the leaf. The principle for the determination of the element base on the specification of the instrument.

Result and Discussion

The result of the qualitative phytochemical analysis is shown and discussed below (Table 1).

Phytochemicals	Result
Alkaloids	++
Flavonoids	++
Saponins	++
Terpenoids	++
Tannins	+
Phenols	+
Steroids	

Table 1: Qualitative Phytochemical Analysis Result.
Absent = -
Moderately Present = +
Highly present = ++

Phytochemical screening of the crude leaf extract of *Corchorus olitorius* was done and the result revealed the presence of Alkaloids, Flavonoids, Saponins, Terpenoid, Phenol, Tannins, and absence of Steroid. These compound are known to show curative activity against several pathogen and therefore could explain its acclaimed used traditionally for the treatment of wide array of illnesses. The phytochemicals present in the leaf may be responsible for preventing disease and promoting health. Findings by some researchers revealed that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity. Phytochemicals may detoxify substances that cause cancer [12]. The phytochemical result corresponded to the acclaimed ethomedical use of the plant. The reported use of the plant leaf ethnomedically in treatment of pains, fever, management of diabetes and hypertension may be due to

the presence of alkaloid, while the reported ability of the leaf to stop dysentery, diarrhea and duodenal tumuor may be as a result of the presence of tannins. The flavonoid in it may serve as antioxidant and free radical scavenger. They reported finding that the leaf is use to neutralize the acidity of inflammations may be due to the presence of (OH) bond in their benzene ring [13] which may also lower cholesterol level in man [14]. The presence of alkaloid in extracts was reported by previous researchers to inhibit the growth of staphylococcus aureus, they are used as antimycotics, and in the treatment of stomach pains [15]. Flavonoids may be responsible for diuretic and antibacterial activity, since they are antioxidants. They may also help in healing of wounds and in treatment of skin diseases due to their ability to neutralize the acidity of wounds and inflammations. They are also used in treatment of diarrhea [13]. Tannin extracts have been reported to be anti-inflammatory, control gastritis and irritating bowel disorder, they may also contribute to antimicrobial power which heals wounds and stop bleeding [15] tannin also ensures the inhibition of organism by coagulating their microprotoplasm [13]. Saponin detected in the plant has been found to be an antibacterial and antimycotic substances on cell wall of many organisms [16] (Table 2).

Phytochemicals	Result
Alkaloids	6.3±0.3%
Flavonoids	6.25±0.5%
Saponins	8.01±0.6%
Tannins	1.03±0.2%
Phenols	1.16±0.01%

Table 2: Quantitative Phytochemical Analysis Result.

The quantitative phytochemical result obtained showed that the different classes of phytochemicals were present in the plant in varying proportions (percentages). Saponin gave highest yield of 8.01% followed by Alkaloid 6.3% and Flavonoid 6.25%, Phenols and tannins where 1.16% and 1.03% respectively. The presence of all these secondary metabolites shows both physiological and medicinal activities [17] (Table 3).

The result of the trace metals (micro nutrients) concentration showed that the leaf of *Corchorus olitorius* contain Chromium, Iron, Manganese, Magnesium, Zinc, Calcium, Iodine, Phosphorus and Potassium in the permissible range by World Health Organization and Standard Organization of Nigeria for food nutrients with the absence of copper. The leaf may be used as remedy for heart failure, disruption of metabolisms and diabetes because of the chromium it contain. It may also be used as remedy for anemia because it contain iron. It may be used in treating glucose intolerance, skin problem, skeleton disorder, birth defects, changes of hair colour, neurological symptoms and fatness because of the manganese it contain. The leaf contain zinc which is used as remedy for loss of appetite, decrease sense of taste and smell, slow wound healing and skin sore and birth defect. The iodine contain in the leaf may be used to prevent struma and skin disease. Therefore the leaf has nutritive value and is recommended for ethno medicine and food supplement (Table 4).

Element	Concentration (mg/kg)
Chromium	0.072
Iron	15
Manganese	4
Iodine	2.4
Phosphorus	16
Calcium	0.1
Magnesium	160
Potassium	150
Copper	0
Zinc	0.88

Table 3: Result of Mineral Element Analysis.

Spot	R_f Value
A	0.13
B	0.22
C	0.38
D	0.63
E	0.8

Table 4: Result of Thin Layer Chromatography of Crude Ethanol Extract (R_f Value).

Thin layer chromatography screening was done using different solvents mixtures on the crude ethanolic extract of the leaf of *Corchorus olitorius* to predict the number of component(s) contained in the leaf and the more appropriate solvents mixture for column chromatography. The solvents mixture that eluted more compound was pet ether 70: 30 Chloroform. The Retardation factor showed a clear separation of five components within the range of 0.13 - 0.80. This corresponded with report that Rf values for natural product always appears as fraction and lies between 0.01 to 0.99 [18] (Table 5).

Pet-ether	Chloroform	Methanol	Solvent	Mixture	Elautes and color
100	0	0	P_{100}		Amber (A) (1A)
90	10	0	P_{90}	C_{10}	-
80	20	0	P_{80}	C_{20}	Yellow (B) (2A)
70	30	0	P_{70}	C_{30}	-
60	40	0	P_{60}	C_{40}	P.Green(C) (3A)
50	50	0	P_{50}	C_{50}	-
40	60	0	P_{40}	C_{60}	-
30	70	0	P_{30}	C_{70}	P.Yellow (D) (4A)
20	80	0	P_{20}	C_{80}	D.Brown (E) (5A)
10	90	0	P_{10}	C_{90}	-
0	100	0	C_{100}	-	-
0	90	10	C_{90}	M_{10}	-
0	80	20	C_{8}	M_{20}	-

Table 5: Result of Column Chromatography.
P = Pet ether = Non polar
C = Chloroform = mid polar
M = Methanol = Polar
Comp = Component

The result showed that the leaf extracts of *Corchorus olitorius* contained more non-polar and mid polar compounds. The first compound eluted at 100% pet-ether as 1A the second eluted at P_{80} C_{20} as 2A. The 3rd compound eluted at P_{40} C_{60} as 3A, while the 4th and 5th eluted at P_{30} C_{70} and P_{20} C_{80} as 4A and 5A respectively (Table 6).

Eluents	*Pseudo*	*Staph*	*E.coli*	*Shigella*	*Strep*
1A	10 mm	5 mm	14 mm	12 mm	-
2A	15 mm	8 mm	3 mm	15 mm	-
3A	10 mm	3 mm	7 mm	17 mm	-
4A	12 mm	2 mm	7 mm	14 mm	-
5A	7 mm	1 mm	5 mm	16 mm	-
Standard	22 mm	32 mm	18 mm	27 mm	-

Table 6: Results of Antibacterial Screening of Column Eluates (Zone of Inhibition) in mm.
Pseudo = Pseudomonas aeruginosa
Staph = Staphylococcus aureus
E.coli = Esherichia coli
Shigella = Shigella species
Strep = Streptococcus pyogenes
Standard = Ciprofloxacin (25mg/ml)

The result of antibacterial screening of column eluates showed that *Shigella* was the most inhibited organism with highest inhibition zone (17 mm) for eluate 3A. 16 mm in 5A, 15 mm in 2A, 14 and 12 mm in eluates 4A and 1A, followed by *Pseudomonas aeruginosa* with the highest zone of inhibition (15 mm) for eluate 2A. *E.coli* has its highest inhibition zone (14 mm) in 1A while *Staphylococcus* has its highest inhibition zone (8 mm) in 2A. *Streptococcus pyogenes* was resistant to the eluates and the antibiotic. These results confirmed that at a higher concentration the leaf extracts will be effective for treatments of the diseases caused by these bacteria that showed high zones of inhibition. The result supported the use of leaf in treatment of dysentery and diarrhea locally due to its inhibition of *shigella* and *E.coli* which are the causal organisms of such diseases [12]. These result support the ethnomedical uses of the plant. The antimicrobial results also supported that of phytochemical which revealed the presence of flavonoid which was reported to exert multiple biological properties including antimicrobial [12] (Table 7).

Eluents	*A. tamarii*	*A. niger*	*A. flavus*	*A. nomius*
1A				
2A			4 mm	
3A				2 mm
4A				
5A				4 mm
Standard	8 mm	8 mm	10 mm	6 mm

Table 7: Results of Antifungal of the Column Eluate (Zone of Inhibition) in mm.
A.tamarii = Asperigillus tamarii
A.niger = Asperigillus niger
A.flavus = Asperigillus flavus
A.nomius = Asperigillus nomius
Standard = Ketoconazole (20mg/ml)

The antifungal screening of the column eluents shows that the leaf will have antifungal activities at higher concentration as it showed some activities at very low concentration, the low concentration of eluate 1A showed no zone of inhibition against the tested organisms. Eluent 2A showed antifungal action against only *Asperigillus flavus* at 4 mm zone of inhibition, 3A showed zone of inhibition against only *Asperigillus nomius* at 2 mm. 4A showed no zone of inhibition against the entire tested organism. 5A showed little zone of inhibition against only *Asperigillus nomius* at 4 mm. The standard showed zone of inhibition against all the tested organisms except *Asperigillus tamari.* These result confirmed that the leaf extract also have antifungal effect.

Conclusion

The results of the study have shown that *Corchorus olitorius* leaf contain some phytochemicals which have antimicrobial activities, these shows that the plant is a medicinal plant. The micro-nutrients analysis confirms that the leaf is rich in mineral elements and may be use as food supplement. The bioassays of the active eluates showed that they have broad spectrum antimicrobial activities comparable to the standard drugs such as ciprofloxacin and Ketoconazole used as positive control. The observed antimicrobial effect of *Corchorus olitorius* leaf extracts appears interesting and promising. This implies that the plant extract may be indeed effective in management of pneumonia and diarrhea as the extract inhibited the growth of *pseudomonas aeruginosa and esherichia coli* which are the casual organisms of the illnesses. It may be use in managing, skin sore, birth defect, anemia, heart failure, neurological symptoms and skeleton disorder due to the presence of some mineral element like Iron, Manganese, Zinc, Potassium, Magnesium and Calcium hence supporting its ethno-medical use. This investigation has scientifically justified the use of *Corchorus olitorius* leaf in ethnomedical practice provided it would be administered within the appropriate toxicity level for human. This research conformed to the findings of previous researchers supporting its ethno-medical use [19,20]. The leaf to the best of my knowledge can provide target for the synthesis of new antimicrobial agent.

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