

Phytochemical Analysis and Antimicrobial Activities of *Cyperus rotundus* and *Typha latifolia* Reeds Plants from Lugari Region of Western Kenya

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Rec date: October 24, 2017; Acc date: November 01, 2017; Pub date: November 06, 2017

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Abstract

Phytochemical compounds and antimicrobial activity of *Cyperus rotundus* and *Typha latifolia* reeds plants from Western Kenya, which are used in management and treatment of various ailments, were determined. Samples were collected from Lugari region of Western Kenya. Solvent extraction using ethyl acetate on powdered samples was done. Five phytochemicals were tested positively in *C. rotundus* and *T. latifolia* this include Tannins, Steroid, Saponin, Alkaloid and Glycoside. Flavonoid was tested negatively in *C. rotundus* and *T. latifolia*. Disc agar diffusion method was adopted for antimicrobial activity test against gram positive and gram negative bacterial. The maximum antimicrobial activities were observed at 100% concentration than at 10% of methanol extracts of *C. rotundus* and *T. latifolia* and no antimicrobial activities observed at 1%.

Keywords: Phytochemicals; Antimicrobial; *C. rotundus*; *T. latifolia* reeds

Introduction

World Health Organization (WHO) has stated that 80% of the world's population depends on traditional medicine for primary health care [1]. Man depends on plants as a source of medicines for treatment of varied ailments as plants are the main precursors for drug development [2]. The large numbers of original medicinal plants are used as food additive and for treatment purpose [3]. These plants contain physiologically active components or phytochemicals which over the years have been exploited in the traditional medical practices for the treatment of various ailments [2]. The most active secondary metabolites of plants are steroids, flavonoids, alkaloids, tannins, saponin and glycosides are active phytochemicals of plants. Some secondary metabolites like alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants.

Phytochemicals enhance flavor and color and also offer protection against diseases by coordinating with nutrients and fibre. Phytochemicals plays critical roles in the body, include boosting the immune system, and inhibit growth of bacterial, reducing inflammation, prevention of cancer and cardiovascular diseases. It is important to know the structure of phytochemical constituents, thus knowing the type of biological activity which might be exhibited by the plant. The antioxidative phytochemicals have received increasing attention for their potential role in preventing/reducing the risk of human diseases such as coronary heart disease. It is believed that if antioxidative phytochemicals are incorporated into the diet, it could lead to beneficial physiological changes in human microflora, which could contribute to their chemo-preventive effects. Of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes) present in active extracts, tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption. Others act by inhibiting intestinal motility.

Phytochemicals are isolated and extracted through different techniques are then screened for their biological activity.

Resistance to antimicrobial agents is an emerging issue in a wide variety of pathogens and multiple drugs, resistance is becoming common in organism such Salmonella and staphylococcus [4]. The appearance of resistant enhanced the occurrence of infectious diseases that are only treated by a limited number of antimicrobials agents [5]. Rise in resistant of bacterial and fungal for antibiotics, antifungal highlights the need to find alternative sources from medicinal plants [6]. Medicinal plants are the sources of many important drugs of the modern world. Many people rely on herbal remedies for their health care because they are cheaper and easily available to most people in developing countries compared to synthetic medicine and has minimum side effect after use. These reasons might account for their worldwide attention and use.

In Western parts of Kenya, *Cyperus rotundus* and *Typha latifolia* are used in treatment of infectious diseases. *Typha latifolia* is broadleaf cattail, an aquatic emergent perennial, morphological and physiological, ecotypes as well as hybrids make field identification difficult. The leaves of broadleaf cattail are mixed with oil and used as poultices on sores and wounds. Pollen is astringent, diuretic, emmenagogue, haemostatis, refrigerant sedative and vulnerary. The dried pollen is said to be anticoagulant, but when roasted with charcoal it becomes homeostatic. It is used internally in treatment of kidney stone, hemorrhages, painful menstruation, abnormal uterine bleeding, postpartum pain, abscesses and cancer of lymphatic system. It should not be prescribed for pregnant women. A decoction of stem has been used in the treatment of whooping cough. The roots are diuretic, galactagogue, refrigerant and tonic. Flowers are used in the treatment of wide range of ailments such as abdominal pains, *Amenorrhoea cystitis*, dysuria and vaginitis. The young flowers heads are eaten as treatment for diarrhea. The seed down has been used as a dressing on burns and scalds.

C. rotundus has a broad spectrum of applications as herbal remedies in China, Africa, Latin, America, India, Saudi Arabia, Sudan and

Kenya. In Asian countries, its rhizomes are used for the treatment of spasms, stomach disorders, bowel disorders and inflammatory diseases. In Chinese pharmacopoeia, it was used to regulate circulation, normalize menstruation, and Relieve pain. In Sudan its tubers are used in stomach disorders and bowels irritation. An infusion of tubers is used in dyspepsia, diarrhea, dysentery, ascites, vomiting, cholera and fevers. The tubers are given in large doses as an anthelmintic. A poultices of the fresh tubers is used to cure wounds, ulcers and sores; it is also applied to the breast to promote the flow of milk. Paste is used in scorpion stings. Despite *C. rotundus* and *T. latifolia* being used in treatment of infectious diseases in Lugari region of Western Kenya, their phytochemical values and antimicrobial activities are not exhaustively done due to lack of knowledge and techniques. The study aimed at phytochemical analysis and antimicrobial activities of *C. rotundus* and *T. latifolia* as are used to treat various diseases in Western Kenya. The crude extracts obtained from *C. rotundus* and *T. latifolia* reeds plants were screened for phytochemicals and antimicrobial activity since their active ingredients and antimicrobial activity have not been exhaustively done.

Materials and Methods

Chemical reagents

In the present study, all the chemicals were purchased from HIMEDIA Pvt. Ltd., Bombay. The chemicals used were of analytical grade.

Collection and identification of plant materials

Samples of *Cyperus rotundus* and *Typha latifolia* reeds plants were collected from Lugari, western Kenya. Plants samples were packed in samples bags, labeled and transported to Botany laboratory at university of Kabanga for scientific classification and later were transferred to chemistry laboratory for preparations, extractions and analysis were carried out. Samples were dried at room temperature, ground into a fine powder using laboratory grinding mill. The fine powder was packed and stored in a cool and dry place for future use in phytochemical research.

Extract preparation

The extraction of each sample was prepared by mixing with solvent of 1:10 (plant sample: solvent). The extraction was done successively using ethyl acetate where each plant sample were soaked in 200 mL solvent in conical flask for 3 days. The extracts were filtered using Whatman No 1 filter papers and solvent removed through evaporation under reduced pressure kept at 45°C using a rotatory evaporator. The extracts were kept in stoppered sample vials at 4°C until they were used.

Preliminary qualitative phytochemical screening

Phytochemical tests were conducted on the extracts of *Cyperus papyrus* and *Typha latifolia* using standard methods as reported elsewhere [7-10]. By this analysis, the presence of several phytochemical like alkaloids, flavonoids, tannins, saponins, steroids and glycosides were tested.

Alkaloids: 2 mL of ethyl acetate extracts of each samples were dissolved in 2 mL of hydrochloric acid and 2 mL of potassium iodide was added, red brown color formed indicated presence of alkaloid.

Flavonoid: About 2 mL of ethyl acetate extracts of each samples were dissolved in 2 ml sodium hydroxide and few drops of hydrochloric acid were added, colorless precipitate formed indicated presence of Flavonoid.

Glycoside: About 2 mL of each ethyl acetate extract of samples were dissolved in 1 mL glacial acetic acid and few drops of concentrated sulphuric acid were added, a brown ring formed indicated presence of glycoside.

Steroids: About 2 ml chloroform was added to the 2 mL of ethyl acetate extracts of each samples, then followed few drops of H₂SO₄ were added to form a lower layer. A reddish-brown color at interface indicated presence of steroidal ring.

Saponin: About 2 mL of the ethyl acetate extracts of each of the samples were dissolved to the 2 mL of distilled water and then shaken for 10 minutes, foam formed indicated presence of saponin.

Tannins: Distilled water measuring 2 mL was added to 2 mL of ethyl acetate extract of each the samples the few drops of FeCl₃ solution was added, green precipitate formed indicated presence of Tannins.

Test of the extracts for antimicrobial activity

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts. 2 mL of a standardized bacterial stock suspension were thoroughly mixed with 200 mL of a sterile molten nutrient agar. 20 ml aliquots of the inoculated nutrient agar will be distributed in sterile Petri dishes. The agar was left to set and three cups (10 mm in diameter) were cut using a sterile cork Borer (no. 4) in each plate and the agar discs were removed using a sterile wire loop. Each cup was filled with 100 µ of one of extracts concentration using microliter pipette and the extract were allowed to diffuse at room temperature for 2 hrs. Two replicates were carried out for each extract against each of the bacterial organisms. The plates were then incubated at 37°C for 18 hrs. Simultaneously, in separate Petri dishes a cup were made for each organism. After incubation, the diameters of the growth inhibition zones were measured, and the average values were tabulated [11-15].

Results and Discussion

Qualitative test

Plant sample/ phytochemicals	<i>C. rotundus</i>	<i>T. latifolia</i>
Saponin	+	+
Steroid	+	+
Tannin	+	+
Alkaloid	+	+
Flavonoid	-	-
Glycoside	+	+

Table 1: Phytochemical test. Where; Key: + indicate presence and - indicate absence.

The Table 1 presents the qualitative test analyses carried out on the ethyl acetate extracts of *Cyperus rotundus* and *Typha latifolia* and show the presence or absence of various phytochemicals.

Phytochemical test revealed the presence of Steroid, saponin, Alkaloid, Glycoside and Tannin in both *C. rotundus* and *T. latifolia*. Flavonoid was absent in extracts of *C. rotundus* and *T. latifolia*.

Antimicrobial assay

The methanol extracts of *C. rotundus* and *T. latifolia* were subjected to antibacterial assay on *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*.

Zone of growth inhibition of *C. rotundus* and *T. latifolia*: Zone of growth inhibition of *C. rotundus* was done and results are tabulated (Table 2). The maximum antimicrobial activities were observed at concentration of 100% of extract than at 10%. No antibacterial activities observed at 1%. The extract was found effective against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* and *Escherichia coli* and was found ineffective against *Staphylococcus aureus*.

Results for zone of growth inhibition of *T. latifolia* are represented in Table 2. The maximum antimicrobial activities were observed at concentration of 100% of extract than at 10%. No antibacterial activities observed at 1%. Among the five bacterial organisms maximum growth inhibition was observed in *Escherichia coli* and *Staphylococcus aureus*. The extract does not inhibit the growth of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*.

Zone of growth inhibition of standard antibiotics: Zone of growth inhibition of standard antibiotics were also done, and results are represented in Table 3. Standard antibiotics such as Gentamicin, Norfloxacin and ceftriaxone have shown maximum growth inhibition against *Pseudomonas aeruginosa* while they are ineffective against *Bacillus subtilis*.

Inhibition of <i>C. rotundus</i>					
Concentration (%)	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>
100	9	8.33	-	10	8.33
10	7.667	7	-	8	-
1	-	-	-	-	-
Inhibition of <i>T. latifolia</i>					
Concentration (%)	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>
100	-	8.67	8	-	-
10	-	7	-	-	-
1	-	-	-	-	-

Table 2: Inhibition of *C. rotundus* and Inhibition of *T. latifolia*.

Antibiotics standards are most effective against bacteria compared to *T. latifolia* and *C. rotundus*. However, *C. rotundus* has shown maximum growth inhibition against *Bacillus subtilis* with an inhibition of 8 at 10% concentration unlike the standard antibiotics and *T. latifolia*. The antibacterial activity index of *C. rotundus* extract ranged from 0.38 (in *Pseudomonas aeruginosa* with Ceftriaxone) to

0.76 (in *Candida albicans* with Norfloxacin) and of *T. latifolia* extract ranged from 0.6 (in *Escherichia coli* with Ceftriaxone) to 0.80 (in *Staphylococcus aureus* with Nalidixic acid). Standard antibiotics such as Gentamicin, Norfloxacin and ceftriaxone have shown maximum growth inhibition against *Pseudomonas aeruginosa* and all are ineffective against *Bacillus subtilis*.

Antibiotics	Concentration	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>
Amoxyclav	20/10 mcg	-	-		-	-
Nitrofurantoin	200 mcg	-	-	11	-	-
Nalidixic acid	30 mcg	-	-	10	-	-
Gentamicin	10 mcg	13	-	11	-	-
Norfloxacin	10 mcg	22	-		-	11
Ofloxacin	10 mcg	-	12	13	-	18
Ceftriaxone	30 mcg	24	14	18	-	-

Table 3: Inhibition of Antibiotics.

Conclusion and Recommendations

C. rotundus have a potential to act as a good antimicrobial agent against *pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* and *Escherichia coli* because of presence of various phytochemical ingredients Steroid, saponin, Alkaloid, Glycoside and Tannin. *C. rotundus* had shown maximum growth inhibition against *Bacillus subtilis* with an inhibition of 8 at 10% concentration unlike the standard antibiotics and *T. latifolia*. *T. latifolia* has shown its effectiveness against *Escherichia coli* and *Staphylococcus aureus* because of the presence of various secondary metabolites such as Steroid, saponin, Alkaloid, Glycoside and Tannin. These phytochemicals seemed to be active source of useful drugs. From this study's findings, *T. latifolia* and *C. rotundus* extracts can be used as good antibacterial agents. *C. rotundus* can be used against *Bacillus subtilis* unlike the standard antibiotics and *T. latifolia*. Further research for isolation and characterization of compounds responsible for the observed antimicrobial activity. There is need to carry out antifungal and antioxidant activities of *T. latifolia* and *C. rotundus*.

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