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TOPICAL ANTI-INFLAMMATORY EFFECT AND HPLC FINGERPRINT OF ETHANOL EXTRACT OF THE RHIZOME OF *COSTUS SPECTABILIS* (FENZL) K. SCHUM

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ABSTRACT

Background: Aqueous extract of *Costus spectabilis* (Fenzl) K. Schum (Costaceae) has been used traditionally as remedy for arthritis and rheumatism.
Methods: Extract was screened for the different classes of phyto-constituents using standard methods. HPLC analysis was developed to establish the fingerprint of the extract, while the topical anti-inflammatory study was evaluated following xylene-induced ear oedema model in mice.
Results: Topical application of xylene to both surfaces of the ear caused inflammation as indicated by increased thickness of the ear and mass of the ear punches. Extract (5 mg/ml) significantly ($p \leq 0.01$) decreased mean increase in the ear thickness comparable to the standard anti-inflammatory drug, indomethacin (0.5 mg/ml). Also, extract (5 mg/ml) and indomethacin (0.5 mg/ml) inhibited inflammation by 76% and 68%, respectively. Additionally, *C. spectabilis* extract conferred a dose-dependent decrease in mass of ear punches. Doses of 5 and 2.5 mg/ml elicited greater inhibition ($p \leq 0.001$) of the initial increase in mass of the ear punches in the control group than indomethacin. The phytochemical constituents of the extract were identified as flavonoids, saponins, steroids and triterpenes, with saponins being most abundant (457.2 mg/g). Furthermore, HPLC fingerprint profile of the extract was established.
Conclusion: Extract of *C. spectabilis* demonstrated topical anti-inflammatory effect in xylene-induced ear oedema model in mice and this could be due to one or some of the phytometabolites.

Key words: *Costus spectabilis*, HPLC fingerprint, anti-inflammatory activity, xylene-induced oedema, saponins.

INTRODUCTION

Costus spectabilis (Fenzl) K. Schum (Costaceae), is a rhizomatous geophyte native to tropical Africa, commonly called Yellow trumpet and 'Takalmin zomo' (in Hausa). The rhizomatous plant thrives in shade. However, certain other species can also tolerate full sunlight in moist soil and humid climates [1]. *C. spectabilis* leaves have been employed in African traditional medicine for the treatment of various illnesses such as internal and external wounds, arthritis, rheumatism, maternal and neonatal infections in Mali [2, 3]. It is also used traditionally as a remedy for cough, as laxative, purgative and diuretic [2].

Inflammation is a physiologic defense response that protects the body against infection, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation is a major symptom of some chronic illnesses such as cancer, cardiovascular diseases, arthritis, inflammatory bowel syndrome, atherosclerosis and autoimmune diseases [4,5,6]. Although widely utilised, the present day anti-inflammatory medications are associated with numerous adverse effects, especially when employed for an extended duration in the management of persistent ailments such as arthritis. Hence, it is crucial to explore new anti-inflammatory agents that could potentially possess greater efficacy and improved safety profile, particularly from plants such as *C. spectabilis*. In this study, an attempt was made to establish the chemical profile and evaluate the topical anti-inflammatory property of the aqueous ethanol extract of *C. spectabilis* rhizome.

MATERIALS AND METHOD

Collection, identification and preparation of plant material

Whole plant of *C. spectabilis* was collected from Shika, Zaria, Kaduna State and authenticated (voucher No. 1611) at the Bioresources Unit, National Research Institute for Chemical Technology (NARICT) Basawa, Zaria by a taxonomist, Mal. U.S Gallah. Rhizome was separated, sliced, shade-dried, pulverized, weighed and packed in a plastic container.

Extraction of plant material

Powdered rhizome (470 g) was macerated in a glass jar with 2.5 L of aqueous ethanol (70% $^{v}/_{v}$) at room temperature for 72 hrs. Extract was filtered through a cotton plug and finally through Whatman no.1 paper. Marc was exhaustively re-extracted for 72hrs. Combined filtrates were concentrated on a rotavapor, dried residue weighed, and kept in a desiccator.

Preliminary phytochemical screening

The aqueous ethanol extract was screened for the presence of different classes of phytochemicals using standard method [7].

Determination of total phenolic content

Estimation of total phenol content in the extract was measured spectrophotometrically (1001Plus, Milton Roy USA, 765 nm) by Folin–Ciocalteu colorimetric method, using gallic acid as the standard according to previous reports [8, 9]. The results were expressed as gallic acid equivalent (GAE) per gram of sample. Different concentrations (0.005-0.025 mg/ml) of gallic acid were prepared in methanol. Aliquots of 0.5 ml (0.025 mg/ml) of the extract and each sample of the standard solution were taken, mixed with 2 ml Folin–Ciocalteu reagent (1:10 in deionised water) and 4 ml saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubated at room temperature for 30 minutes with intermittent shaking. Absorbance (UV-Spectrophotometer 1001Plus, Milton Roy

USA) was taken at 765 nm using methanol as blank. All the samples were analysed in three replicates. Total phenol was determined from standard curve of gallic acid.

Determination of total flavonoid content

The total flavonoid content of *C. spectabilis* rhizome extract was determined by aluminium chloride colorimetric assay [10]. Aliquot (0.5 ml) of the extract (0.1 mg/ml) and a standard solution of quercetin (0.02-0.1 mg/ml) were separately mixed with 2 ml of distilled water. Thereafter, 0.15 ml 5% sodium nitrite solution was added to each mixture, followed by 0.15 ml 10% aluminium chloride solution after 6 minutes. Mixtures were allowed to stand for another 6 minutes before adding 2 ml 4% sodium hydroxide solution. The final volume was made up to 5 ml with distilled water, mixed thoroughly, and allowed to stand for an additional 15 minutes. Absorbance of each mixture was then measured spectrophotometrically (UV-Spectrophotometer 1001Plus, Milton Roy USA) at 510 nm against the same mixture, and the total flavonoid content calculated as mg quercetin equivalent per gram of extract using the quercetin calibration curve. The determinations were done in triplicate.

Determination of saponin content

Total saponin content was determined according to a previously described method [11]. The freeze-dried plant extract was dissolved in 50% aqueous methanol and 1ml aliquot (0.1 mg/ml) taken. Vanillin reagent (0.25 ml; 8%) was added followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixture was mixed thoroughly, incubated at 60°C in a water bath for 10 min, cooled on ice and absorbance read at 544 nm (UV visible spectrophotometer) against a blank. Initial standard calibration curve was obtained from absorbance/concentrations of varying dilutions of the standard sapogenin, diosgenin (0.5 mg/ml in 50% aqueous methanol). The total saponin concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW) of the extract.

HPLC analysis

The chromatographic separation of compounds from ethanol extract of the rhizome *C. spectabilis* was conducted based on established method [12] using Agilent technology 6495 Triple Quad LCT/MS/HPLC-Alliance 2695-watermaslynx LCT-MS equipped with an Agilent Zobrax C18 column (4.6 x 150 mm, 5 µm) from Agilent Technologies. Acidified water (0.1 % formic acid, v/v) and methanol were used as mobile phases A and B respectively. The gradient elution was programmed as follows: 0 min, 70% A and 30% B; 30 min, 100% B. Flow rate was set at 1 ml/min throughout the elution, the injection volume was 10 µL and the column temperature was maintained at 25˚C.

Determination of topical anti-inflammatory activity

***Animals*:** Thirty albino mice (18–22 g) of either sex were used for the study. The mice were obtained from the animal house stock of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. Animals were handled in accordance with the guidelines as per the ABU Zaria Animal Ethical Committee. The study commenced following approval by the University Animal Use and Care Committee with approval number ABUCAUC/2017/001.

Xylene-induced ear oedema model in mice: Topical anti-inflammatory activity of the extract using xylene induced ear oedema in mice was carried out following standard method [14]. Mice were divided into six groups of 5 animals each, and the negative control group received normal saline only. Oedema was induced in each mouse in the

treated groups by topical application of xylene (30 µL) to both inner and outer ear surfaces. After 15 minutes, 0.1 ml 70% ethanolic solutions of extract (1.25, 2.50 and 5 mg/ear) and indomethacin in normal saline (0.5 mg), the positive control, were applied to inner and outer surfaces of the right ear. The thickness of each ear was measured using a Vernia caliper before induction of oedema and at thirty minutes after xylene application. Animals were sacrificed under chloroform anesthesia, equal size of the ear pinna was cut off, and weighed.

Statistical Analysis

The results were expressed as mean ± SEM and were analyzed using one-way ANOVA followed by Dunnett's post hoc test. Statistical significance was set at $p \leq 0.05$.

RESULTS

Phytochemical screening

The phytochemical screening of *C. spectabilis* rhizome aqueous-ethanol extract revealed the presence of various classes of secondary metabolites (Table 1). The quantity of some of the constituents identified are as presented (Table 2).

Table 1: Phytochemical screening of *Costus spectabilis* rhizome extract

Phytochemical Constituent	Inference
Phenolic compounds	+
Flavonoids	+
Steroids	+
Triterpenes	+
Saponins	+

Key: + signifies presence

HPLC profile

The HPLC chromatogram of the extract revealed sixteen peaks with retention times (R_t) ranging between 1.24 and 29.94 min. Most prominent peaks occurred at R_t: 1.24, 1.62, 1.96, 3.77, 25.54, 27.17, 27.94 and 29.94 (Fig. 1).

Table 2: Quantitative estimation of classes of secondary metabolites

Secondary metabolite	weight (mg/g extract)
Phenolic compounds	31.0
Flavonoids	12.7
Saponins	457.2

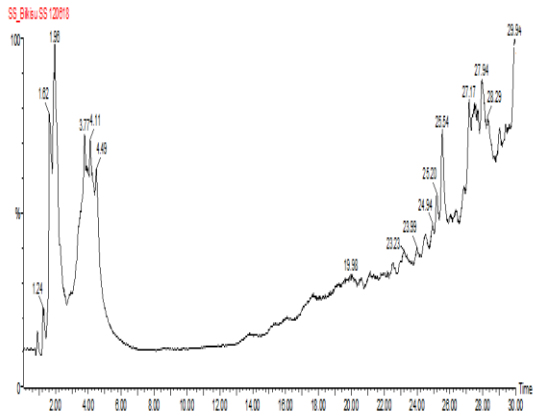

Figure 1: HPLC chromatogram of ethanol extract of *Costus spectabilis* rhizome

Topical anti-inflammatory effect

C. spectabilis extract conferred significant topical anti-inflammatory effect measured as reduction in thickness of the xylene-induced ear edema. Highest dose of the extract (5 mg/ml) produced significant ($p < 0.01$) reduction comparable to indomethacin, the standard anti-inflammatory drug used. Similarly, the percent increase in ear thickness of 69.44% observed in the control group was significantly and comparably reduced by 5 mg/ml of extract and indomethacin to 11.70% and 12.90%, respectively. Lower doses of extract (2.5 and 1.25 mg/ml) conferred lower effect (Table 3 and Figure 2). Evaluation of activity based on reduction in weights of the ear punches

produced effect comparable to indomethacin in a dose-dependent manner (Table 4).

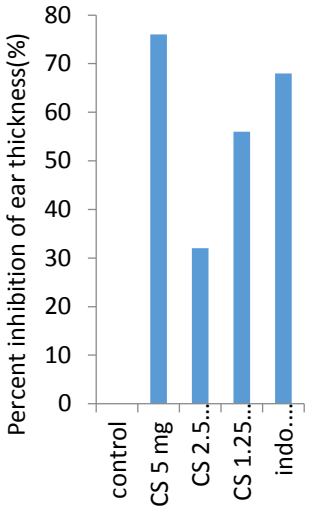

Figure 2: Inhibition (%) of inflammation by *Costus spectabilis* extract (CS) and indomethacin (Indo)

Table 4: Effect of ethanol extract of *Costus spectabilis* on xylene-induced increase in weights of ear punches

Treatment	Weight of ear punch (mg) ±SEM
Untreated control	21±1.80
CS (5 mg/ml)	11±0.44***
CS (2.5 mg/ml)	12.4±1.07***
CS (1.25 mg/ml)	14.2±1.82**
Indomethacin (0.5 mg/ml)	13.8±1.20**

Values are Mean±SEM., *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ compared to untreated control group were considered significant, n = 6. Key: CS = *Costus spectabilis* 5, 2 and 1.25 mg/ml extract group.

Table 3. Effect of *Costus spectabilis* extract on xylene-induced ear oedema in mice

Group/ dose	Final thickness (mm)	Initial thickness (mm)	Mean increase in thickness (mm) ± SEM	Percentage (%) increase in thickness
Normal control	0.119±0.030	0.119±0.030	-	-
Untreated control	0.1525±0.023	0.090±0.027	0.0625±0.008	69.44
CS (5 mg/ml)	0.1425±0.022	0.1275±0.028	0.0150±0.007**	11.76
CS (2.5 mg/ml)	0.1150±0.006	0.0725±0.006	0.0425±0.009	58.62
CS (1.25 mg/ml)	0.1175±0.024	0.090±0.0270	0.0275±0.005*	30.55
Indomethacin (0.5 mg)	0.1750±0.006	0.1550±0.015	0.0200±0.011**	12.90

Values are Mean ± S.E.M., *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ compared to untreated control group were considered significant, n = 6. Key: CS = *Costus spectabilis* 5, 2 and 1.25 mg/ml extract group.

DISCUSSION

This study has revealed the different classes of phytochemicals that are present in the rhizome of *C. spectabilis*. Previous studies have identified flavonoids, coumarins, alkaloids, sterols, triterpenes, reducing compounds, oses and holosides from *C. spectabilis* leaf [14]. Furthermore, several other reports of the presence of sterols [15], steroidal saponins [16], flavonoids and flavonoid glycosides in *Costus* species abound [17]. Result of quantitative estimation indicated saponin as the major class of phytochemicals in the extract. Many saponins were known to accumulate in the rhizomes of closely related species, like *C. speciosus* [17] and *C. afer* [19].

Quality control of herbal medicines has been an important concern for both health authorities and the public because of their wide usage [20]. Chromatographic fingerprints of herbal medicines indicate their chemical characteristics and can be used to determine their identity, consistency and authenticity [21]. Consequently, HPLC chromatographic fingerprinting has become one of the most useful approaches in quality control of herbal medicines [22]. The HPLC fingerprint developed in this study under established analytical conditions can be used as a characteristic spectrum of *C. spectabilis*. Previous studies have reported HPLC fingerprints of several other *Costus* species such as *C. igneus* [23], *C. pictus* [24], and *C. speciosus* [25], but under different HPLC conditions.

The findings of this present study showed that extract of *C. spectabilis* rhizome exhibited significant topical anti-inflammatory effect. Several *Costus* species are known to have anti-inflammatory properties. For example, extracts of *C. speciosus* rhizome and *C. pictus* leaf have been found to reduce both paw oedema and leukocyte migration [26, 27]. Similarly, *C. afer* stem extract and *C. igneus* rhizome extract have been shown to inhibit the production of pro-inflammatory cytokines, in addition to reducing paw oedema and leukocyte migration [27, 28]. Furthermore, methanol extract of *C. afer* rhizome was shown to exert significant topical anti-inflammatory effect in croton aldehyde-induced mouse ear oedema, while its chloroform extract ameliorated all signs associated with adjuvant-induced polyarthritis in rats [29].

Many inflammatory compounds isolated from various *Costus* species include 5-hydroxy-7-methoxyflavone from *C. pictus* rhizome [30], 3β-hydroxycostic acid, a diterpene from *C. speciosus* leaf known to exert significant anti-inflammatory activity by inhibiting the production of nitric oxide (NO) and prostaglandin E2 (PGE2) [31]. Likewise, costunolide isolated from *C. speciosus* rhizome, has been shown to attenuate the expression of tumor necrosis factor alpha (TNF α), interleukin (IL), IL 6, inducible NO synthase (NOS), and cyclooxygenase (COX 2) [32]. Furthermore, diosgenin isolated from *C. speciosus* has demonstrated a highly significant inhibitory effect on TNF α [33], and β-amyrin isolated from *C. igneus* has been found to significantly inhibit PGE2, IL-6 secretion, and NF-κB [34].

Xylene-induced ear edema model is useful for the evaluation of topical anti-inflammatory steroids and steroidal anti-phlogistic agents, especially those inhibiting phospholipase A_2 [35]. Application of xylene induces acute neurogenous edema, which is particularly associated with substance P. Substance P is found throughout the central and peripheral nervous system. When sensory neurons release it in the periphery, it causes vasodilation and plasma extravasations, resulting in swelling of the ear. This suggests that xylene plays a role in neurogenic inflammation [36]. The ear edema associated with xylene involves inflammatory mediators, including histamine, kinin, and fibrinolysin [37]. The extract significantly inhibited ear swelling in mice, indicating that its active components may reduce the release of substance P or other inflammatory mediators such as histamine, kinin, and fibrinolysin, or counteract their effects.

CONCLUSION

C. spectabilis rhizome aqueous-ethanol demonstrated topical anti-inflammatory effect against xylene-induced ear edema in

mice, and this could be due to one or some of the chemical constituents identified.

Conflict of interest

Authors declare no conflict of interest.

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