Original Article

Evaluation of Nutraceutical content and topical Anti-inflammatory Activities of *Turraea vogelii* Hook F. Ex Benth (Meliaceae)

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ABSTRACT

Background: *Turraea vogelii* has been used as traditional medicine for the treatment of ailments including wounds, stomach aches, malaria, infections, filariasis cutaneous, among others and, it serves as source of food. The present study aimed to evaluate the nutraceuticals content and topical anti-inflammatory effect of hydro-methanol twig extract of *Turraea vogelii* (METV).

Method: METV was obtained using cold maceration with water and methanol in ratio 30:70. The concentration of vitamins A, C and E, total phenolic and total flavonoid contents were evaluated by UV spectrophotometric method. Phenol and xylene-induced ear edema was used to evaluate the topical anti-inflammatory effect in mice.

Results: The concentration of vitamins A, C and E was found to be 0.113±0.006, 9.303±0.012 and 0.020±0.001 mg in 100g of dried plant materials, respectively. Total phenolic and flavonoid contents were 0.281±0.318 mg/g gallic acid equivalent and 0.112±0.269 mg/g quercetin equivalent, respectively. Topical application of METV at a dose of 25 and 50mg/ear in xylene-induced ear edema produced significant inhibition (P<0.05) of 33 and 54% while in phenol-induced ear edema was significantly (P< 0.05) reduced by 44% and 34%. Dexamethasone (0.1mg/ear) produced an inhibition of 83% and 57% in phenol-induced ear edema.

Conclusion: Results from this study demonstrated that *Turraea vogelii* twigs contain flavonoids and phenolic compounds, which may be responsible for the topical anti-inflammatory effect of the plant extract.

Keywords: Anti-Inflammatory Effect, Dexamethasone, Phenol, Topical, *Turraea Vogelii* Extract, Xylene.

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INTRODUCTION

A nutraceutical is described as a food or part of a food with medical or health benefits, or as a substance with physiological benefit which protects against chronic diseases (1). Nutraceuticals are believed to improve health, delay aging process, prevent chronic diseases, increase life expectancy and support the structure and function of the body, among others (2). They possess numerous therapeutic effects, such as immune enhancement (3) antioxidant (4) cardiovascular (5) and anti-inflammatory (6) effects. Common nutraceuticals with multiple therapeutic properties include natural herbs like ginseng, Echinacea, green tea, glucosamine, omega-3, lutein, folic acid, and cod liver oil (7).

The skin as the principal physical barrier to the external environment provides an important bodily defence mechanism when subjected to injury and invasion by pathogens or other external noxious agents (8). Normally, this defence mechanism aims to repair the tissue damage or destroy the invading pathogen; however, an inappropriate or misdirected immune activity can be implicated in the pathogenesis of a large variety of inflammatory skin disorders, such as psoriasis and atopic dermatitis (9). Thus, a variety of medicinal plants have been widely explored in folk medicine for the topical treatment of various inflammatory or related conditions, especially various forms of dermatitis (10). Herbal nutraceuticals with anti-inflammatory effect include Gentianine present in Gentian root, Bromolain, a proteolytic enzyme found in extracts of stinging nettle, turmeric and the extracts, pineapple and teas (7).

The merits of topical drugs are: *a)* able to by-pass the first-pass hepatic metabolism; *b)* convenient to use;

c) able to achieve efficacy with a lower total daily dose; *d)* simple to discontinue when required; *e)* and site-specific and have stable drug levels with improved adherence (11). Also, topical agents are capable of inhibiting the expression of cytokines, growth factors, adhesion molecules, necrosis factor-κB (NF-κB), nitric oxide, prostanoids and other autacoids as well as inhibit prostaglandins production (12).

Turraea vogelii is used for nutritional purposes and in traditional medicine for treatment of various ailments including wound healing, stomach aches, malaria, intestinal worms and urogenital infections (13). It is used against cutaneous and subcutaneous parasitic infections, filariasis, as laxative and tonic, and it is also used as a ritual plant in Kenya (14). In Central African Republic, the *T. vogelii* leaves are used to treat snake bites and intestinal worms (15). Recent studies have reported its anti-proliferative activity against cancer cells

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(16) and oral anti-inflammatory and anti-nociceptive properties (17). The traditional and medicinal uses of *T. vogelii* for the treatment of wounds, cutaneous and subcutaneous parasitic infections serve as an important basis for the evaluation of the topical anti-inflammatory effect of this plant. The aim of this study was to evaluate the nutraceutical contents and topical anti-inflammatory effect of hydro-methanol extracts of the twigs of *T. vogelii*.

MATERIALS AND METHODS

Animals

Twenty male Swiss albino mice (20-25g) were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria. Ethical clearance was obtained from the University of Ilorin Ethics Review Committee with approval number UERC/ASN/2018/1111. All experiments were carried out in accordance with the Guidelines for laboratory procedures set by the University of Ilorin Ethics Committee on Research as well as the International Animal Care and Use Committee (IACUC) in Nigeria.

Collection and Identification of Plant Material

The fresh leaves of *T. vogelii* were collected in January, 2017 from Onigambari Plantation Reserve, Ibadan, Nigeria. Identification and authentication of the plant was done by an expert (Mr. Odewo, SA) from the Forestry Research Institute of Nigeria (FRIN).

Preparation of Hydro-Methanol Leaf Extract

The twigs were separated from the plant and shade dried for 7 days. They were then pulverized, using a milling machine. A 250 g powdered leaves was weighed into a clean jar and macerated in 2 L of methanol and water (70:30) for 48 hours. The mixture was filtered using cotton wool followed by Whatman filter paper (No. 1). The extract was evaporated using rotary vacuum evaporator at controlled temperature. The resultant filtrate was evaporated to dryness on water bath at 40° C. The percentage yield of the filtrate was calculated.

Evaluation of Nutraceutical Contents of Turraea Vogelii

Determination of Vitamin A Concentration

A 0.5 g of plant material was homogenized and saponified with 2.5 mL of 12% alcoholic potassium hydroxide in a water bath at 60 ^{o}C for 30 minutes. The saponified extract was transferred to a separating funnel, containing 10-15 mL of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer, containing the carotenoids, was collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was recorded. The absorbance of the yellow colour was read, using a spectrophotometer at 460 nm (18).

Determination of Vitamin C Concentration

Ascorbate was extracted from 1 g of the plant material using 4% TCA and the sample was taken in a test tube, 2 mL of distilled water was added, mixed thoroughly and allowed to stand for 30 minutes at room temperature. Then tubes were centrifuged at 3000 rpm for 10 minutes. The clear supernatant was taken in a cuvette without disturbing precipitate and the absorbance was measured at 700 nm (19).

Determination of Vitamin E Concentration

To a 1.5 mL of the sample, 1.5 mL of xylene was added then centrifuged. One mL of xylene layer was transferred to a tube and 1 mL of dipyridyl was added. Absorbance of the mixture was measured at 460 nm. To the mixture, 0.3 mL ferric chloride solution was added and the absorbance measured at 520 nm (20).

Determination of Total Phenolics Content

Total phenolics content was estimated according to the method of Makkar *et al.*(21). The aliquot of the extract was taken and 1 mL of distilled water was added. Then 0.5 mL of Folin-ciocalteu reagent and 2.5 mL of sodium carbonate solution was added. The tubes were placed in the dark for 40 minutes and the absorbance was then measured at 725 nm. A standard curve was prepared with Gallic acid monohydrate. The linearity obtained was in the range of 1-10 μg/mL. The standard curve was used to calculate the total phenolic content and expressed as Gallic acid equivalent in mg/g of the extract.

Determination of Flavonoid Content

The aluminium chloride colorimetric assay was used (21). To 1 mL of extract, 4 mL of distilled water was added. To the above mixture, 0.3 mL of 5 % sodium nitrite was added. After 5 minutes, 0.3 mL of 10 % $AlCl_3$ was added. A 2mL of 1 M NaOH was added after 6 minute and made up to 10 mL with distilled water. Absorbance was measured at 510 nm. The total flavonoid content was expressed as percentage of Quercetin equivalent per 100 mg of fresh mass (21).

Determination of Topical Anti-Inflammatory Effect of Turraea Vogelii

The 20 mice were randomly allocated into four groups of five animals; negative control group (normal saline); treatment groups (25 and 50 mg/ear) and dexamethazone (dexa) group (0.1 mg/kg), as the standard drug.

Xylene Induced Mouse Ear Edema

A modified method of Tubaro *et al.* (22) was used. Xylene (20 μL) was applied to the inner and outer surfaces of the right ear of the mice while the left ear received 20 μL of vehicle, acetone. Thirty minutes after induction of edema, the right ear was topically treated with METV (25 and 50 mg/ear, in 20 μL of acetone) and dexamethasone (0.1mg/ear, in 20 μL of acetone positive control for xylene) and acetone (20 μL/ear, negative control). Two hours later, mice were sacrificed and a plug (6 mm in diameter) was removed from both the treated and the untreated ears. The edematous response was measured as weight difference between the two plugs.

Phenol-Induced Mouse Ear Edema

The Gábor method (23) was used with minor modification. Inflammation was induced in mice by applying 20 μL of 10% phenol (v/v) in acetone to the inner and outer surfaces of the right ear. METV (25 and 50 mg/ear in 20μL of acetone) and dexamethasone (0.1 mg/ear in 20 μL of acetone) were applied topically to right ear thirty minutes after the application of phenol. The left ear received 20 μL of acetone only. The ear sample weight was measured and evaluated 2 hours later as described above.

Quantification of Ear Edema

Two hours after treatment, the animals were euthanized under ether anaesthesia and both ears were removed. Circular sections were taken using a cork borer (6 mm diameter) and weighed. Ear edema was determined by comparing the weight (mg) of the sections removed from the right versus the left ear of each mouse. The anti-inflammatory effect was evaluated as the edema reduction (24) in the treated animals compared to controls.

Statistical Analysis

Data was expressed as mean ± standard error of the mean (SEM). The results were analyzed using Graph pad software program version 6.0. Statistical analysis was determined by one way analysis of variance (ANOVA), with the statistical significance set at $p<0.05$.

RESULTS

The total amount of Vitamins A, C and E in 100 g of *T. vogelii* are shown in Table 1. Also, the total amount of phenolic and flavonoid contents in 100 g of the plant material are presented in Table 2.

Table 1. Vitamin contents in the *T. vogelii* twigs.

Vitamin	Amount in mg/100g of plant material
Vitamin A	0.113±0.006
Vitamin C	9.303±0.012
Vitamin E	0.021±0.001

Table 2. Total phenolic and flavonoid contents of the *T. vogelii* twigs.

Constituents	Amount in extract
Total phenolic content (*mg/g* Gallic acid equivalent)	0.281±0.318
Total flavonoid content (*mg/g* Quercetin equivalent)	0.112±0.269

Effect of Hydro-Methanol Extract of T. Vogelii on Xylene-Induced Mice Ear Edema

The hydro-methanol extract of *T. vogelii* exhibited anti-inflammatory activity. Topical application of METV (25 and 50 mg/ear) significantly inhibited the ear edema by 33% and 54%, respectively, as compared to the negative control group ($P<0.05$). Dexamethasone, a steroidal anti-inflammatory drug, produced 83% inhibition of ear edema ($P<0.001$) relative to the controls (Fig. 1).

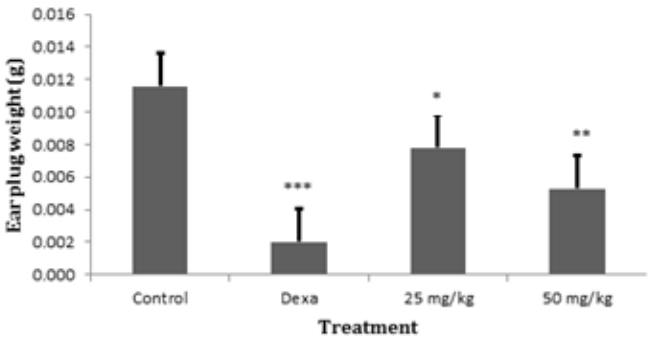

Figure1. Topical effect of METV on xylene–induced mice ear edema, mean ±S.E.M (*n=5).*
*P < 0.05, compared with negative control group.

Effect of Hydro-Methanol Extract of T. Vogelii on Phenol-Induced Mice Ear Edema

Topically, METV (25 mg) significantly ($P < 0.05$) inhibited the phenol-induced ear edema by 44 % while dexamethasone exerted an ear edema inhibition of 57% ($P < 0.01$) as compared to the control. The 50mg extract gave an insignificant edema reduction relative to the negative control (Fig. 2).

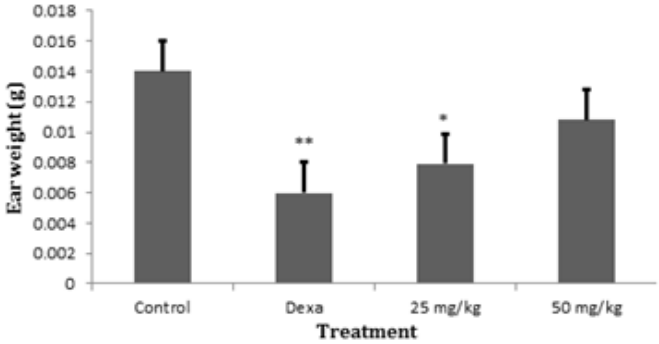

Figure 2. Topical effect of METV on phenol-induced mice ear edema. Mean±SEM (*n=5).*
**P*<0.05 compared with negative control group.

DISCUSSION

Turraea vogelii was found to contain vitamins A, C and E. The amount of vitamin C was higher than vitamin A and E in 100 g of the plant sample. Vitamin C is water-soluble with a five-membered ring polyol. Each hydroxyl group can accept a reactive oxygen species. This gives vitamin C its antioxidant and anti-inflammatory effect (25). The anti-inflammatory effect of the plant may, therefore, be attributed in part to its vitamins A, C, E contents.

Phenolic compounds are used in skin infections, wounds treatment and healing (26). The extract contained a significant amount of phenolic and flavonoid compounds, which are consistent with a previous study (17) that reported the presence of flavonoids, tannins and terpenoids in the extract of the plant. The synergistic effect of vitamin C with flavonoids and other phenols have been correlated with the inhibition of cancer cell proliferation and other inflammatory conditions (27).

Acute inflammation characterized by classical symptoms, such as warmth, redness, swelling and pain is an important response produced by inflammatory agents. Xylene is known to cause local increase in capillary permeability, inflammatory cell infiltration and acute exudative inflammatory edema in mouse ears (28). Acute inflammation induced by xylene is thus frequently used to investigate the anti-inflammatory effects of drugs in mice. This experimental model is used to evaluate both steroidal and non-steroidal anti-inflammatory drugs (29). The inhibition of inflammation produced by xylene is an indication of the anti-inflammatory effect of a putative drug or extract. METV at doses of 25 and 50 mg/ear significantly inhibited ear edema produced by xylene in a dose-dependent manner. The effectiveness of the extract at doses of 25 and 50 mg/ear may suggest the inhibition of phospholipase A, which is involved in the pathogenesis of inflammation (30).

Similarly, phenol-induced inflammation was inhibited by METV at a dose of 25 and 50 mg/ear as compared to that observed in the control group. The significant inhibition of phenol-induced edema, produced at 25 mg/ear, implies that METV may have a potential use in contact dermatitis as phenol has been postulated to be a good irritant agent for simulating contact dermatitis in mice (31). The major action of phenol is on the keratinocyte membrane, which promotes the release of pro-inflammatory mediators and some reactive oxygen species (32). It was observed that the anti-inflammatory effect of METV was not dose-dependent in the phenol-induced group unlike the xylene group that had a dose-dependent activity. This phenomenon is not uncommon in studies involving the use of plant extracts as some previous studies have reported similar findings (33). The inhibitory effect of METV on phenol-induced ear edema may be related to the anti-inflammatory and antioxidant effect of the extract. The appreciable concentration of total phenolic and flavonoids compounds in *T. vogelii* is, therefore, suggestive of its potential antioxidant and anti-inflammatory property.

CONCLUSION

This study demonstrated the topical anti-inflammatory effect of the hydro-methanol extract of the *Turraea vogelii* twigs in mice ear edema, induced by xylene and phenol. The findings justifies its traditional use in skin disorders and also indicates its potential application as a herbal medicine for cutaneous inflammatory conditions.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interests in conducting this study.

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