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Antioxidant, Analgesic and Antipyretic Activities of Ethanol Extract of the Stem Bark *Dichaetanthera africana* (Hooker F.) Jacq. Felix. (Melastomataceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RSP, RANN and ASM designed the study and supervised the work. Author CEG co-supervised the work. Authors ALMK, PP and PNP worked in the laboratory to carry out the experiments. Author ATT prepared the plant extract. Authors GAA, RMEE and RSM helped in preparing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was designed to elucidate the antioxidant, analgesic and antipyretic activities of the ethanol extract of the stem bark of *Dichaetanthera africana*.
Place and Duration of Study: Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, VNS Group of Institutions, Bhopal, India, between September 2014 and November 2014.
Methodology: Antioxidant activities were determined *in vitro* by the total phenolic, total flavonoid, ferric reducing antioxidant power (FRAP), DPPH, hydrogen peroxide and ABTS methods. Analgesic and antipyretic activities were investigated using the acetic acid induced writhing and brewer's yeast induced hyperpyrexia assays respectively.
Results: Ethanol extract had high phenolic (4.15-44.35 mgCA/g) and flavonoid (85.56-596.7 mgCA/g) contents and FRAP (187.87-371.41 mgCA/g). This extract exhibited strong DPPH (IC_{50} = 0.49 µg/ml), H_2O_2 (IC_{50} = 1.62 µg/ml) and ABTS (IC_{50}= 0.43 µg/ml) radical scavenging activities. Significant reduction of abdominal writhing ($P< 0.001$) and fever ($P< 0.001$) were observed in rats in a dose dependent manner after administration of the ethanol extract with the most effective dose being 400 mg/kg b.w.
Conclusion: These results provide promising baseline information for the potential use of *Dichaetanthera africana* in the treatment of pain and fever.

Keywords: Abdominal constriction writhing; fever; Melastomataceae; radical scavenging activity; total flavonoid; total phenol.

1. INTRODUCTION

Oxygen is needed by living organisms for energy production to fuel biological processes [1]. However, there is the risk of the oxygen being transformed to reactive oxygen species which may induce oxidative stress and subsequent damage to biological structures. Reactive oxygen species (ROS) have been associated with some disease conditions such as cancer, diabetes, cardiovascular diseases, atherosclerosis, hypertension, ischemia injury, neurodegenerative diseases, inflammation and ageing [2]. Antioxidant agents have been reported to play an important role in the management of these disease conditions either as prevention or treatment [3]. Pain is an unpleasant sensation which may be beneficial because it is a defensive mechanism of the body during tissues damage [4]. Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer. However, severe pain can arise independently of any obvious predisposed cause or can persist after the precipitated injury has healed and can also occur as a consequence of brain or nerve injury [5]. With many pathological conditions, tissue injury is the immediate cause of the pain, and these results in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation [6]. By acting in the central nervous system or on the peripheral pain mechanism, analgesic compounds selectively relieves pain without significant alteration of consciousness. Analgesics are applied when the noxious stimulus cannot be removed or as adjuvant to more etiological approach to pain [7]. Fever is a regulated increase of body core temperature characterized by an increase thermoregulatory set point, which result from the interaction of the central nervous and immune systems [8]. While fever is a hallmark of injury, infection and inflammation, it has also been considered to be the most important component of a complex host response to invading agents, called acute-phase response [9]. Although there is evidence supporting the idea that fever enhances host defenses, some studies have suggested that raising core temperature to the febrile range may be harmful. Therefore, in clinical situations in which fever-associated risks outweigh benefits, antipyretic treatment is indicated [10]. Most of the antipyretic drugs inhibit cyclooxygenase-2 (COX-2) expression to reduce the elevated body temperature by inhibiting prostaglandin E_2 (PGE_2) biosynthesis. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles, whereas natural COX-2 inhibitors usually have lower selectivity with fewer side effects [11].

Dichaetanthera africana is a tree of 9 to 15 m in high, found in riverine woodland from Sierra Leone to Congo and Angola [12]. The different parts of this plant are medicinally used in Cameroon, Gabon, Sierra Leone, Ivory Coast and Nigeria against coughs, chest pain and fatigue [13,14]. *Dichaetanthera africana* is also used as an ornamental plant thanks to its bright pink flowers. Akoué et al. [15] showed that the ethanol, hydroalcoholic and aqueous extracts of the branches of *Sakersia africana*, an isotype of *Dichaetanthera africana*, had high total phenol content and good antiradical activity. Besides, others investigators have shown that the species of Melastomataceous family exhibited antinociceptive [16], anti-inflammatory, antipyretic [17] and antioxidant [18] activities. Apart from the use of *D. africana* in treatment of inflammation and pain in folk medicine, there is no report about chemical and biological studies on this plant. The present study was designed to evaluate the antioxidant, analgesic and antipyretic properties of ethanol extract of the stem bark of *Dichaetanthera africana*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The stem bark of *D. africana* was collected from the Littoral Region of Cameroon (Babimbi II) in February 2012. Identification was done at the National Herbarium (Yaoundé, Cameroon), where a voucher specimen (No 7157/SRFK) has been deposited.

2.2 Preparation of Crude Extract

The air-dried and powdered stem bark of *D. africana* (2 kg) was macerated at room temperature in ethanol (5 l, 72 h) and then evaporated using a rotary evaporator (Büchi R200) to obtain a crude extract (76.3 g). The yield of the extraction (3.82%, w/w) was calculated with respect to the initial weight of the dry pulverized stem bark.

2.3 Experimental Animals

Sixty six nulliparous and non-pregnant Wistar rats (180–200 g) were used and housed in plastic cages under normal laboratory conditions (12 h light/dark cycle: 25±2℃) for an acclimatization period of 7 days prior to the experiments. All animals were given food and water *ad libitum*.

2.4 Preliminary Phytochemical Screening

Preliminary phytochemical screening was performed according to the methods described by Khandelwal [19].

2.4.1 Identification of alkaloids

The ethanol extract (2 g) was boiled in a water bath with 20 ml of 5% H_2SO_4 in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute H_2SO_4.

2.4.1.1 Mayer's test

To the filtrate in test tube I, 1 ml of Mayer's reagent (potassium mercuric iodide solution) was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids.

2.4.1.2 Dragendoff's test

To the filtrate in test tube II, 1 ml of Dragendoff's reagent (potassium iodide and bismuth nitrate) was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

2.4.2 Identification of glycosides

The extract (2 g) was dissolved with 10 ml of ethanol. The solution was then filtered and the filtrate was divided into three portions for the following tests.

2.4.2.1 Raymond's test

To the test solution, 1 ml of 50% ethanol, 0.1 ml of 1% solution of dinitrobenzene in ethanol and 3 drops of 20% NaOH solution were added. The appearance of violet colour is due to the presence of active methylene group.

2.4.2.2 Killer Killiani's test

Test solution was dissolved in a mixture of 1% $FeCl_3$ solution in (5%) glacial acetic acid. To this

solution, 3 drops of concentrated H_2SO_4 were added. A reddish brown colour appears at the junction of the two liquid layers and upper layer appears bluish green due to the presence of desoxy sugar.

2.4.2.3 Legal test

To the sample solution, 0.1 ml of pyridine, 2 drops of 2% sodium nitroprusside and a drop of 20% NaOH solution were added. A deep red colour is produced.

2.4.3 Identification of carbohydrates

The extract (2 g) was dissolved with 10 ml of ethanol. The solution was then filtered and the filtrate was divided into three portions for the following tests.

2.4.3.1 Molisch's test

Few drops of 20% solution α-naphtol in ethanol were added to the sample solution. This was followed by addition of 1 ml of concentrated H_2SO_4 along the side of the test tube. A violet ring forms at the junction of two liquids.

2.4.3.2 Fehling's test

Equal volumes of Fehling's solution A (copper II sulfate) and Fehling's solution B (potassium sodium tartrate) were added to the sample solution. The mixture was heated for few minutes. The production of yellow or brownish red precipitate of cuprous oxide indicates the presence of reducing sugars.

2.4.3.3 Benedict's test

Two milliliters of Benedict's reagent (anhydrous sodium carbonate + sodium citrate + copper II sulfate pentahydrate) were added to the test sample. The mixture was heated for 5 min. The colour change from blue to green, yellow, orange or red depending upon the amount of reducing sugar present in the test sample.

2.4.4 Identification of tannins

The extract (2 g) was dissolved with 10 ml of ethanol. The solution was then filtered and the filtrate was divided into two portions for the following tests.

2.4.4.1 Vanillin-hydrochloric acid test

One milliliter of vanillin-hydrochloric reagent (vanillin + ethanol + concentrated hydrochloric acid) was added to the sample solution. A pink or red colour forms due to formation of phloroglucinol.

2.4.4.2 Gelatin test

To the sample test, 1 ml of aqueous solution of gelatin and sodium chloride were added. A white buff coloured precipitate forms.

2.4.5 Identification of flavonoids

The extract (2 g) was dissolved with 10 ml of ethanol. The solution was then filtered and the filtrate was divided into two portions for the following tests.

2.4.5.1 Lead acetate test

Filter paper strip was dipped in the sample solution and ammoniated with ammonia solution. A yellow coloured precipitate forms.

2.4.5.2 Shinoda's test

Few magnesium chips and 2 drops of concentrated HCl were added and warmed. A pink or red colour indicates the presence of flavonoids.

2.4.6 Identification of resins

The extract (2 g) was dissolved with 10 ml of ethanol. The solution was then filtered and the filtrate was divided into two portions for the following tests.

2.4.6.1 Ferric chloride test

Few drops of 1% $FeCl_3$ solution were added in the test sample. A green colour indicates the presence of resins.

2.4.6.2 Turbidity test

The test sample was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of resins.

2.4.7 Identification of steroids

The extract (2 g) was placed in a test tube and dissolved with 10 ml of ethanol. The mixture was heated for 3 min. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating dish to

dryness and 5 ml of petroleum ether was added to the dish and stirred for 5 min, the petroleum ether portion was then decanted and discarded. Ten milliliters of chloroform was then added and stirred for about 5 min, it was then transferred into test tube and 0.5 mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests.

2.4.7.1 Lieberman-Burchard's reaction

To test tube I, 1 ml of 4% NaOH solution and 3 drops of 1% copper sulphate solution were added and gently mixed. The appearance of red or violet colour indicates the presence of steroids.

2.4.7.2 Salwoski's test

To test tube II, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 were added and mixed to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of steroidal ring.

2.4.8 Identification of proteins and amino-acids

The extract (2 g) was dissolved with 10 ml of ethanol. The solution was then filtered and the filtrate was divided into three portions for the following tests.

2.4.8.1 Biuret test

To the sample solution, an equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution were added. The appearance of violet colour indicates the presence of protein.

2.4.8.2 Precipitation test

The sample solution was mixed with absolute alcohol. The appearance of white indicates the presence of proteins.

2.4.8.3 Ninhydrin test

Two drops of freshly prepared 0.2% Ninhydrin reagent was added to the test sample. The mixture was heated for 10 min in water bath. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino-acids.

2.5 *In vitro* Antioxidant Activity

The *in vitro* antioxidant activity was characterized by total phenolic content (TPC), total flavonoid content (TFC), Ferric reducing antioxidant power (FRAP), Free radical scavenging activity (DPPH), hydrogen peroxide scavenging activity and ABTS scavenging activity. All analyses were performed in triplicates with appropriate reference standards. TPC, TFC and FRAP were expressed as milligram catechin (CA) per gram of dry weight. The antioxidant activity of the sample expressed as IC_{50} values was defined as concentration (µg/ml) of sample that inhibits the formation of DPPH, H_2O_2 and ABTS radicals by 50%. All values were obtained by interpolation from linear regression analysis.

2.5.1 Total phenolic content

TPC was estimated by the Folin–Ciocalteu method [20]. Twenty microliters of extract (0.625-10 mg/ml) was added to 0.4 N Folin–Ciocalteu reagent (980 µl). Catechin (10–80 µM) was used as a standard for the calibration curve. Absorbance at 760 nm was measured after 5 min of incubation at room temperature.

2.5.2 Total flavonoid content

TFC was determined by the colorimetric method [21]. The sample of extract (0.1 ml; 0.625-10 mg/ml) was mixed with 5% (w/v) sodium nitrite (0.2 ml). After 5 min, 10% (w/v) aluminum chloride (0.2 ml) and 1M NaOH (2 ml) were added. Catechin (50–600 µM) was used as a standard for the calibration curve. Absorbance was measured at 510 nm.

2.5.3 Ferric reducing antioxidant power (FRAP)

FRAP was performed by the method of Benzie and Strain [22]. FRAP reagent was prepared with 300 mM acetate buffer pH±3.6 (100 ml), 10 mM 2,4,6-tripyridyl-s-triazine (10 ml) and 20 mM $FeCl_3$, H_2O (10 ml). Seventy five microliters of extract (0.625-10 mg/ml) was added to FRAP reagent (2 ml) and the mixture was incubated at room temperature for 15 min. Catechin (50–600 µM) was used as a standard for the calibration curve. Absorbance was measured at 593 nm.

2.5.4 DPPH free radical scavenging

Free radical scavenging activity of the extract on the stable radical DPPH was estimated by the method of Mensor et al. [23]. One hundred microliters of extract (0.25-4 mg/ml) was mixed with 1900 µl of DPPH methanol solution (30 mg/l) and kept for 30 min at room temperature in

a dark cupboard. The decrease in the solution absorbance, due to proton donating of substances was measured at 517 nm. L-Ascorbic acid (15.62-250 µg/ml) was used as positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

2.5.5 Hydrogen peroxide radical scavenging

Hydrogen peroxide scavenging activity of the extract was determined using the method described by Rutch et al. [24]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH7.4). Hydrogen peroxide solution (0.6 ml) was mixed with 1.4 ml of extract (0.625-10 mg/ml). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min of incubation against blank solution containing phosphate buffer without hydrogen peroxide. L-Ascorbic acid (10-80 µg/ml) was used as reference standard. The percentage of H_2O_2 scavenging activity was calculated using the following formula:

H_2O_2 scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

2.5.6 ABTS radical scavenging

2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid) ($ABTS^+$) scavenging effect of the extract were analyzed in accordance with the method of Re et al. [25]. The ABTS radical was generated by mixing equal volumes of 7 mM of ABTS and 4.9 mM of potassium permanganate (KMnO4) and kept in the dark room for 24 h. Eight milliliters of the ABTS generated radical solution was diluted further in 72 ml of distilled water. One ml of the diluted radical solution was added to 20 µl of plant extract (0.625-10 mg/ml) and the optical density read at 734 nm after 12 min of incubation. L-Ascorbic acid (15.62-250 µg/ml) was used as positive control.

ABTS scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

2.6 Acute Oral Toxicity

The test for acute toxicity was carried out using the OECD guidelines for acute toxicity [26]. Animals were divided into 3 groups of 6 animals each. The control group was orally administered water (10 ml/kg b.w.) while test groups were administered doses of extract (1000 and 2000 mg/kg b.w). All the animals were observed for toxic manifestations for the next 5 h and subsequently observed intermittently for signs of morbidity and mortality for 7 days. No animal died even at 2000 mg/kg. Hence one-tenth (200 mg/kg b.w.) and one-fifth (400 mg/kg b.w.) of this dose were selected as therapeutic doses to investigate analgesic and antipyretic activities.

2.7 Analgesic Activity

The peripheral analgesic activity of the extract was assessed in acetic acid induced abdominal writhing experiments using Albino rats (24 rats). The standard procedure prescribed by Veerappan et al. [27] was used for observing the abdominal constriction writhing resulting from intraperitoneal injection of acetic acid (10 ml/kg of 0.6% v/v glacial acetic acid solution in normal saline). Four groups of 6 rats each were orally treated distilled water (10 ml/kg b.w), sodium diclofenac (100 mg/kg b.w) and extract (200 and 400 mg/kg b.w). Acetic acid solution was then administered to each animal after 30 min and the number of writhing counted for the next 15 min. The percentage inhibition of writhing was calculated by using the following formula:

Inhibition of writhing (%) = $[(M_{control} - M_{sample} / M_{control})] \times 100$

Where

M= Mean number of writhing

2.8 Antipyretic Activity

The estimation of antipyretic efficacy of the extract was carried out using brewer's yeast induced pyrexia method [28]. Fever was induced by means of subcutaneously injecting 10 ml/kg of a 20% w/v suspension of Brewer's yeast in normal saline. Only animals whose rectal temperature increased by at least 0.5℃ after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a digital thermometer. Experimental animals (24 rats) were randomly divided into four groups containing six animals in each group and received orally distilled water (10 ml/kg b.w), aspirin (100 mg/kg b.w) and extract (200 and 400 mg/kg b.w). The rectal temperature was recorded at time intervals of 1, 2, 3, 4 and 5 h after drug administration. Rectal temperature reduction for

each treatment was calculated in arbitrary unit as the area under the curve (AUC).

2.9 Statistical Analysis

Data were expressed as mean±SD. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test. The 16.0 SPSS Windows software was used for this analysis. Differences between values were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening revealed the presence of alkaloids, glycosides, carbohydrates, tannins, flavonoids, resins and steroids (Table 1). Identification of bioactive principles from medicinal plants is crucial for the standardization of herbal drugs. Other investigators [29,30] have reported the presence of these components in the Melastomataceous family to which belongs *D. africana*. In addition, Akoue et al. [15] working on the branches of *Sakersia africana*, an isotype of *D. africana*, revealed also the presence of these compounds. The alkaloids, tannins, flavonoids are known to possess antioxidant and analgesic properties in various studies [31,32,33].

3.2 Antioxidant Activity

TPC, TFC and FRAP of *D. africana* increased in a concentration dependant manner (Fig. 1). TPC and TFC were found in the range of 4.15 to 44.35 mgCA/g and 85.56 to 596.7 mgCA/g respectively. FRAP of extract was found in the range of 187.87 to 371.41 mgCA/g. TPC of the extract was relatively low compared to TFC and FRAP. *D. africana* exhibited good H_2O_2, DPPH and ABTS scavenging activities with IC_{50} values of 1.62, 0.49 and 0.43 µg/ml respectively (Table 2) although weak when compared to those of ascorbic acid (0.05 and 0.63 µg/ml). However, the antiradical activities obtained by DPPH and ABTS methods were simular. These activities were stronger than those obtained by H_2O_2 method. Flavonoid and phenolic compounds are known to act as antioxidants, radical scavengers, metal chelators, mediators and inhibitors of enzymes [34]. The capacity of phenolics to scavenge free radicals may be due to many hydroxyl groups they possess [35]. Phenols play an important role in antioxidant activity, because they transfer hydrogen to radicals and produce phenoxide radical, which is stabilized [36]. Scavenging activities of *D. africana* may be due to the presence of these bioactive constituents. Some studies have demonstrated a relationship between phenolic content and antioxidant capacity [37]. Antioxidant activity increased proportionally to the phenolic content [38].

Table 1. Qualitative analysis of phytoconstituents of the ethanol extract of *D. Africana*

Phytoconstituents	Identification tests	Observations
Alkaloids	Dragendorff's test	+
	Mayer's test	+
Flavonoids	Lead acetate test	+++
	Shinoda's test	+++
Glycosides	Raymond's test	+++
	Killer killani's test	+++
	Legal test	+++
Carbohydrates	Molisch's test	+++
	Fehling's test	+++
	Benedict's test	+++
Tannins	Vanillin-HCl test	+
	Gelatin test	+
Resins	Ferric chhloride test	++
	Turbidity test	++
Steroids	Liebermann-buchard's test	+
	Salkowski's test	+
Proteins and amino-acids	Biuret test	-
	Precipitation test	-
	Ninhydrin test	-

-: absent, +: present at low concentration, ++: present at moderate concentration, +++: present at high concentration

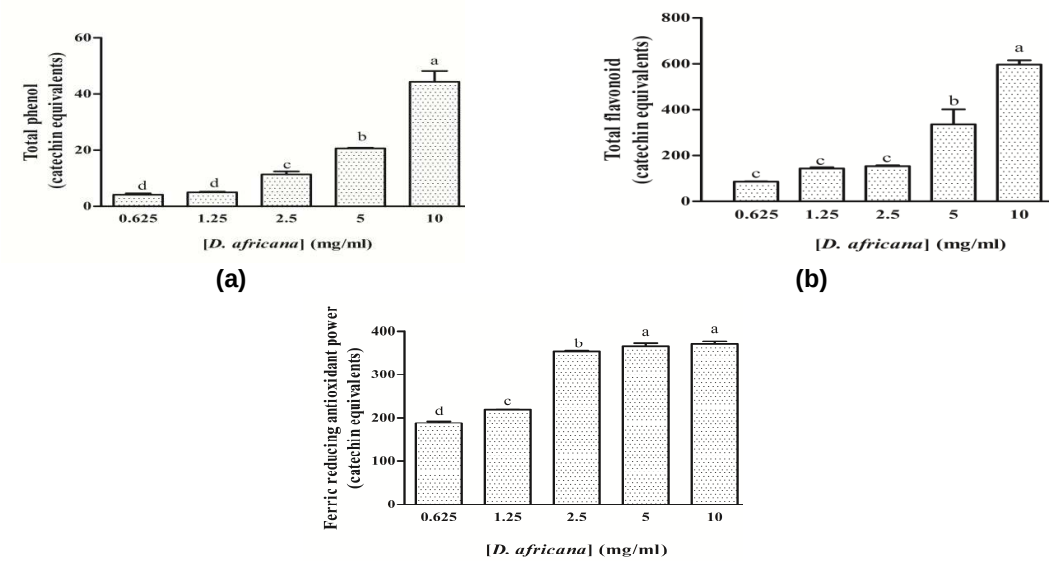


Fig. 1. Total phenol (a), total flavonoid (b) and ferric reducing antioxidant power (c) present in ethanol extract of *D. africana*, expressed as catechin equivalent

[a,b,c,d]For the different concentration, values carrying different letters in superscript are significantly different at P< 0 .05 (Newman-Keuls test)

Table 2. DPPH H_2O_2 and ABTS scavenging activities of the ethanol extract of *D. africana* (IC_{50})

Sample	DPPH (µg/ml)	H_2O_2 (µg/ml)	ABTS (µg/ml)
Ethanol extract of *D. africana*	0.49±0.02[b]	1.62±0.05[b]	0.43±0.04[b]
Ascorbic acid	0.05±0.03[a]	0.63±0.02[a]	0.05±0.02[a]

[a,b] In the same column, values carrying different letters in superscript are significantly different at P< 0.05 (Newman-Keuls)

3.3 Acute Oral Toxicity

In the 7 days LD_{50} determination test, no adverse reactions or mortality were observed at 1000 and 2000 mg/kg oral administration of ethanol extract (LD_{50} > 2000 mg/kg). Hence extract may not be toxic [26].

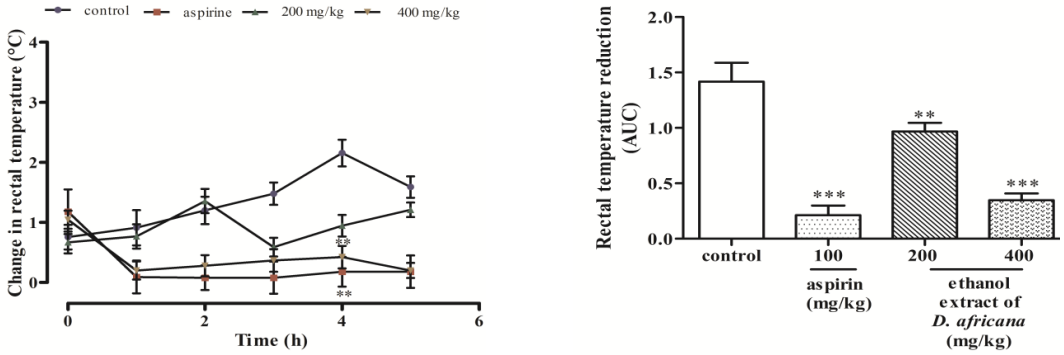
3.4 Analgesic Activity

The oral administration of both doses (200 and 400 mg/kg b.w) of the extract significantly (P< 0.001) inhibited the acetic acid-induced abdominal writhing in rats in a dose dependent manner with a maximum inhibitory response (71.49%) at the dose of 400 mg/kg b.w. (Table 3). *D. africana* showed at 400 mg/kg b.w, a better activity than sodium diclofenac (65.70%). The writhing test has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of plant extracts and natural products. It has been suggested that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons [39]. It is postulated that the abdominal constriction response is induced by local peritoneal receptor activation [40] and involves prostanoids mediators. In rats, there is an increase in the peritoneal fluid levels of PGE2 and PGF-2, as well as lipooxygenase products [41], release of sympathetic nervous system mediators [42]. The nociceptive properties of acetic acid might also be due to the release of cytokines, such as NF-kB, TNF-α, interleukin-1β, and interleukin-8, by resident peritoneal macrophages and mast cells [43]. The inhibitory effects of *D. africana* might therefore to be due to interference with the activation of nociceptors by one of these endogenous mediators or the suppression of sensitization of nociceptors to prostaglandins.

Table 3. Effect of the ethanol extract of *D. africana* on acetic acid-induced rat writhing reflex

Treatment	Dose (mg/kg)	Number of writhing	% inhibition
Acetic acid	—	41.4±9.81	0
Sodium diclofenac	100	14.2±2.77***	65.70
Ethanol extract of	200	21.6±4.44***	47.82
D. africana	400	11.8±6.01***	71.49

Significant difference (Newman-Keuls): compared to control (water); (*) – P< 0.05, (**) – P< 0.01, (***) – P< 0.001; n=6



(a) **(b)**

Fig. 2. Effect of the ethanol extract of *D. africana* on time-course curves (a) and rectal temperature reduction (b) in the protocol of the brewer's yeast-induced rat pyrexia

**P<0.05; **P<0.01; ***P<0.001 compared to control (Newman-Keuls); n=6*

3.5 Antipyretic Activity

The experimental rats showed a mean increase of about 0.93℃ in rectal temperature after 18 h of yeast injection (Fig. 2 above). In rats treated with the standard drug (aspirin), rectal temperatures of experimental animals were coming back to normal after 5 h. The extract at the dose of 400 mg/kg b.w showed good antipyretic effect (P< 0.001) and comparable to that of aspirin when compared to the control group. There had been a sharp decrease in elevated rectal temperature after 1 h of extract administration at the dose of 400 mg/kg b.w. which was further followed by a gradual trend of decrease in a dose dependent manner up to 5 h. Yeast-induced fever or pyrexia is also called pathogenic fever and its etiology could be the production of prostaglandins which set the thermoregulatory center to increase body temperature [44]. Yeast induced pyrexia is considered as a useful test for the screening of synthetic drugs as well as plant extracts for their antipyretic activity [45]. The hypothermic activity of *D. africana* might have been achieved by its action on COX-2 thereby reducing the concentration of PGE-2 in the brain or by enhancing the inherent production of the body's own antipyretic substances such as arginine and vasopressin [46]. An alternative could have been by vasodilation of superficial blood vessels leading to increased dissipation of heat as a result of a reset of the hypothalamic temperature control centre [47]. Flavonoids are known to target prostaglandins which are involved in pyrexia [48].

4. CONCLUSION

The results of the present study indicate that the ethanol extract of *Dichaetanthera africana* exhibits interesting antioxidant properties, as well as significant analgesic and antipyretic effect purported to be mediated via peripheral and central mechanisms which might be attributed to the presence of biologically active compounds. This could provide a rationale for the use of this plant in treatment of pain and fever in folk medicine.

CONSENT

It is not applicable.

ETHICAL ISSUE

Yes, the permission to use rats. "Animal care and handling was done according to the Committee

for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). This guideline has been issued by the Ministry of Environment and Forests, Government of India. Animal study was performed at VNS Group of Institutions, Faculty of Pharmacy, Bhopal (Madhya Pradesh) with due permission from Institutional Animal Ethics Committee (Registration No. 778/PO/a/03/CPCSEA; 03.09.)."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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