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Antidiarrheal, Antispasmodic and Antioxidant Properties of Extracts of Conyza Canadensis

Jamila El-Akhal*, Mohamed El Kabbaoui, Boukir Abdelatif, Tazi Abdelali, Bencheikh Rachid

Department of Science, Sidi Mohamed Ben Abdellah University, Fez, Morocco

****Corresponding author:*** *Jamila El-Akhal, Department of Science, Sidi Mohamed Ben Abdellah University, Fez, Morocco. E-mail:* jamilaelakhal@usmba.ac.ma

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ABSTRACT

Introduction: *Conyza Canadensis is a medicinal plant widely used in traditional medicine in Morocco for the treatment of several health challenges. The present study aimed to investigate antidiarrheal, antispasmodic and antioxidant properties of aqueous methanolic and hydromethanolic extracts of C. Canadensis, to provide data on their mechanisms of action on Wistar rats and to reveal the main functional groups using Fourier Transform Infra-Red Spectroscopy (FTIR).*

Methods: *Antidiarrheal activity of aqueous and methanol extracts of C. Canadensis was assessed on Wistar rats at the doses of 50, 100 and 200 mg/kg body weight using castor oil-induced diarrhea, castor oil-induced enter pooling and small intestinal transit models. The Hydromethanolic extract was studied in the isolated rat jejunum, for antispasmodic property, using KCl (100 mM) or acetylcholine (Ach 10 µM) and in the absence and the presence of L-NAME (100 µM). DPPH and FRAP were used to study the antioxidant activity of aqueous extract. The functional chemical groups were determined by FTIR.*

Results: *C. Canadensis extracts inhibited antidiarrheal activity. The hydromethanolic extract caused a marked concentration-dependent relaxation in rats jejunum pre-contracted by acetylcholine (10 µM) or KCl (100 mM). The NO pathway activation seems not to be involved in the spasmolytic effect C. Canadensis exhibited powerful antioxidant potentiality using DPPH and FRAP as compared with gallic acid and BHT. Ketone, phenol and ester were found to be the main functional groups in C. Canadensis.*

Conclusions: *Our results strongly suggest that C. Canadensis presents promising natural source for the treatment of gastrointestinal disorders.*

Keywords: Conyza Canadensis; Enteropooling; Transit intestinal; DPPH; FRAP; L-NAME; FTIR.

INTRODUCTION

Many people still rely on traditional healing practices and still have a strong belief on medicinal plants despite developing revolutionary technology and huge improvements in healthcare [1]. Many natural products have been described to possess therapeutic benefits, however many others remain partially or fully untested and their use are either poorly monitored or not even monitored at all [2]. Conyza Canadensis is a good example of a plant where the claimed therapeutic effects do not relay in any scientific evidence.

C. Canadensis belongs to well-known family of the Asteraceae. This annual, biennial or perennial plant seems to like to grow in very damp ground and in ground submerged in fresh water, it rarely shrubs, growing to 1-2 m tall. The stems are erect, branched, with alternate leaves. The flowers are produced in inflorescences, with several inflorescences loosely clustered on each stem.

C. Canadensis has been reported to have massive biological activities, encompassing, antiproliferative activity [3] gastrointestinal symptoms, most commonly diarrhoea and dysentery, and as diuretic agent [4]. It also reported to be useful to treat internal haemorrhages, gonorrhea and bleeding piles [5]. Literature survey revealed that the whole plant is antirheumatic, astringent, balsamic, emmenagogue, styptic, tonic and vermifuge [6]. Its leaves are experimentally hypoglycemic [7] .C. Canadensis was also reported to exhibit a significant anti-inflammatory effect on rats with a carrageenin and formalin oedema [8].

The present study is designed to investigate the antidiarrheal and antispasmodic effects of aqueous, methanolic or hydromethanolic extracts of C. Canadensis and to investigate potential mechanisms of action. It is known that oxidative stress can influence responses of the gastrointestinal tract and thus, be the reason of several diseases including the gastrointestinal troubles like spasm and diarrhea [9-10]. Therefore, the antioxidative effect of C. Canadensis extracts is also described in the present study.

Furthermore, an association between the plants used to prepare the present extracts and the presence of functional groups in C. Canadensis was performed using Fourier Transform Infra-Red Spectroscopy (FTIR) with the aim to allow a better standardization of the plants used in folk medicine for the prevention or the treatment of gastrointestinal diseases in Morocco.

MATERIALS AND METHODS

Plant material and preparation of extracts

Aerial parts (leaves and flowers) of C. Canadensis were harvested from their natural habitat at Taounate, a northern province of Morocco, in March 2018. The authentication of C. Canadensis was done based on its macroscopic by Professor Khalid Derraz (Faculty of Sciences and Technologies, Fez, Morocco). A voucher specimen was taxonomically identified and deposited in the herbarium of the Faculty of Sciences and Technologies, Fez, Morocco (Nº: MA-FSTF33).

The fresh herbs were thoroughly washed individually under running tap water to remove any traces of soil particles and other dirt and dried in shade. The dried plant material was powdered using a mechanical grinder place prior to use. The plant extract was prepared following the standard traditional method described in Moroccan Pharmacopoeia [11]. Air-dried aerial parts of C. Canadensis (20 g) were added to 200 mL distilled water to get aqueous extract (AECC); to 200 ml of methanol to get methanolic extract (MECC); or to 70% distilled water and 30% methanol to prepare hydromethanolic extract (HMECC). They were agitated for 24h and filtered. The filtrate of the plant extracts was centrifuged, frozen at -20°C and then lyophilized. The percentage yields based on the dried starting materials was 28 % for C. Canadensis for dried HMECC. The extracts were stored at 4°C until the pharmacological assays were performed.

In vivo experiments

Acute toxicity studies of AECC and MECC on rats

The acute toxicity of the C. Canadensis extracts AECC and MECC was evaluated according to the Organization for Economic Cooperation and Development (OECD) guideline 423 (OECD, 2001a) [12]. Wistar rats (150 g, 250 g) were randomly divided into different experimental groups of six rats each (three males and three females / group). The animals were fasted overnight prior to the experiment. AECC was administered orally by gavage (10 ml/kg) to each treatment group, at single doses of 800, 2000, 3000, 5000 mg/kg, respectively. In order to reduce the number of animals needed, the acute toxicity study of MECC was assessed using single dose of 2000 mg/kg). A control group was given distilled water (10 ml/kg. The animals were allowed food and water ad libitum and kept under regular observation for mortality and behavioral responses within and after 24h following administration, in order to observe the general signs and symptoms of toxicity or mortality.

Castor oil-induced diarrhea

A group of 48 Wistar rats were fasted for 24 hours prior to the experiment, given water ad libitum and divided into eight groups of six rats each (n=6). Group I was given distilled water 10ml/kg body weight (negative control), group II 50mg/kg of AECC or MECC, group III 100 mg/kg of AECC or 100 mg/kg of MECC, group IV 200 mg/kg of EACC or 200 mg/kg of MECC and group V received 5 mg/kg of the standard positive control loperamide. One hour after, castor-oil (3 ml/kg) was administered to each rat. The treated rats were then housed in separate clean cages having paper placed below for collection of fecal matter [13]. The paper was changed every hour for a total of four hours.

Following castor oil administration parameters such as onset of diarrhoea, weight of wet stools, and total number of faecal output were recorded within a time frame of four hours and compared with those of the control. The percent inhibition of defecation and the percentage of faecal output (POF) were measured using the following formulae:

$$POI = [(Mo-M)/Mo] \times 100$$

Where Mo= Mean defecation of control and M=Mean defecation of test sample and POI is percentage of inhibition.

$$POF = (Ft/Fc) \times 100$$

Ft: The mean fecal weight of each treatment group.

Fc: The mean fecal weight of the control group.

POF: Percentage of faecal output.

Small intestinal transit time

Wistar rats were randomly allocated to eight groups of sex rats each. The group I was given normal saline 10 ml/kg body weight (control); group II, III, IV were treated with 50 mg/kg, 100 mg/kg, 200 mg/kg of AECC, and V, VI and VII with 50 mg/kg, 100 mg/kg, 200 mg/kg of MECC; group VIII was given loperamide 5 mg/kg (positive control). Castor-oil (3 ml/kg) was administered to each rat one hour after extract or control treatments. One hour after castor oil administration, animals were feed with 3ml/kg of the marker diet (10% charcoal suspension in 5% cellulose) orally, by gavage. The animals were sacrificed one hour after the charcoal meal, by inhalational anesthesia, using chloroform. The small intestine, from pylorus to caecum, was immediately isolated. The peristaltic index (PI; *i.e.* the distance traveled by the charcoal meal relative to the total length of small intestine, expressed in %) was calculated for each rat using the following equation:

$$\% IP = (LM / LSI) \times 100$$

Where IP = peristaltic index

LM= Length of charcoal meal

LSI= Length of small intestine

Castor oil–induced enter pooling

Intraluminal fluid accumulation was determined following the protocol described by Robert et al [14]. Overnight fasted rats (150 g, 250 g) were divided randomly into eight groups of six animals and treated with 10 ml/kg of distilled water (control); with extracts or with loperamide, as described (section 2.2.3). One hour after, animals were treated with 3 mL of castor oil, orally, to produce diarrhea. One hour after castor oil administration, the animals were sacrificed, the abdomen of each rat was opened; the small intestine was then taken from the pylorus to the caecum, ligated at both ends and dissected out carefully and weighed. Thereafter, its content was collected, by gentle milking into a graduated tube, and the volume of intestinal contents measured and each intestine reweighed. The difference between the full and the empty intestines was calculated. The percentage inhibitions of the volume and weight of intestinal contents were determined according to the following formulae:

$$POI = [(MVICC)-(MVICT)] \times 100/ (MVICC)$$

Where, MVICC = Mean volume of the intestinal content of the control group; MVICT = Mean volume of the intestinal content of the test group.

***In vitro* experiments**

Wistar rats of both sexes, weighing between 200 g and 250 g, were euthanized by decapitation and segments of jejunum, of 2-3 cm, were quickly isolated and mounted in 10 ml organ baths containing warmed (37°C) and oxygenated Krebs-Henseleit (composition, in mM): 69 NaCl; 3.6 KCl; 2.8 CaCl2; 2.96 MgSO4; 1.65 NaH2PO4; 2.1 NaHCO3; 11.2 EDTA; 52.8 glucose; 2.2 ascorbic acid, kept at pH 7.4. Each segment was fixed to a myograph under an optimal tension of 1.5 g.

Each segment of isolated jejunum was allowed to equilibrate and stabilize in normal Krebs solution for 60 min, renewed every 10 min, before the addition of any drug. The segments were used once and changed after the administration of each drug. The isolated jejunum preparations were precontracted with acetylcholine (10 µM). The spasmolytic effect of C. Canadensis (0.003 mg/ml - 3 mg/ml) was tested in the absence or in the presence of 100 µM L-NAME (a nitric oxide synthase inhibitor).

Effect of HMECC, on ach or KCl-evoked contractions in isolated rat jejunum

Each piece of jejunum segment was allowed to equilibrate and stabilize in normal Krebs solution, during 60 min, before the addition of any drug, with washout every 10 min. Thereafter, different concentrations of HMECC (0.003-3 mg/ml) were added cumulatively on acetylcholine- or KCl-evoked contractions. The relaxations of HMECC extracts were expressed as percentage of maximal relaxation (return to base line position).

Effect of L-NAME on the relaxant effect induced by HMECC on ach-precontracted rat jejunum preparations

In order to evaluate whether the effect of HMECC involves the NO pathway, the jejunum segment was incubated for 20 min in the presence of 100 µM L-NAME (a specific NO synthase inhibitor) and cumulative concentrations of the extract (0.003-3 mg/ml) were added to the organ bath.

Antioxidant activity

Antioxidant activity of AECC was determined using two commonly accepted assays, namely DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay and the Ferric Reducing Antioxidant Potential (FRAP). Butylated hydroxytoluene (BHT) and gallic acid were used as references.

DPPH assay

The antioxidant activity of the AECC was assessed by their ability to scavenging DPPH stable radicals, by using the method described earlier by Boudkhili et al. [15]. AECC (0.015^{-1} mg/ml) was added to 2 ml of a 0.004 % MeOH solution of DPPH. The mixture was shaken vigorously and kept at room temperature, protected from light, for 30 min. Absorbance of the AECC and the standards, gallic acid and BHT were measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation from:

$$DPPH\ activity = (A0- A1)/ A0 \times 100$$

Where A0 is the absorbance of the control, and A1 is the absorbance of the extract

FRAP assay

The reducing power of AECC was determined according to the method previously described [16]. AECC (0.05-1 mg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M; pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K3Fe(CN)6]. Thereafter, the mixture was incubated at 50°C for, 30 min. afterwards; 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm, for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%), and the absorbance measured at 700 nm. Increased

absorbance of the reaction mixture indicated increased reducing power.

FTIR analysis

Fourier Transform Infra-Red (FTIR) analysis was performed with a spectrometer (Jasco FT/IR spectrometer series 6800 c in order to find out the characteristic functional chemical groups present in the C. Canadensis components. The spectrum was recorded in transmittance mode at the region between 4000 and 600 cm^{-1}, with a spectral resolution of 4 cm^{-1} and collected with 32 scans. The analysis was carried out at room temperature and ambient humidity.

Drugs

Loperamide, acetylcholine, EDTA, castor oil, charcoal, DPPH (full name), methylene blue were all purchased from Sigma Chemicals Company (St Louis, MO, USA). All the drugs were dissolved in distilled water. Fresh solutions were prepared for each experiment.

ETHICAL APPROVAL

This study was carried out in accordance with the guideline of the Institutional Animal Care and Use Committee at Faculty of Sciences and Technology (FST), USMBA (N°. FST-IACUC-2017-2) and of the European Communities Council Directive (86/609/EEC), with the approval of the Ethics Committee (reference No 255/2018/ORBEA, March 8, 2019.

STATISTICAL ANALYSIS

The data are expressed as means ± SEM (standard error of mean). The data were analyzed using one-way ANOVA followed by Tukey and student test for comparing the control and the various groups, using Graph Pad Prism version 6.1 (Graph-Pad Software, San Diego, California , USA). Statistical significance was assumed at the 0.05 levels.

RESULTS

***In vivo* experiments**

Acute oral toxicity test

AECC and MECC showed no sign of acute toxicity in rats up to 24 hours following oral administration. No deaths were recorded even at the highest dose of 5000 mg/kg body weight for AECC and at 2000 mg/kg body weight for MECC. Therefore, the LD50 of C. Canadensis extracts was assumed to be higher than 5000mg/kg, indicating a wider safety margin.

Castor oil–induced diarrhea

Oral administration of AECC and MECC at the doses up to 200 mg/kg body weight leads to a reduction in the frequency of defecation, fecal dropping, and the mean weight of feces and delayed the onset time, when compared with the untreated controls. At the dose of 200 mg /kg, AECC and MECC provide up to 93.2 ± 6.8% and 100% reduction of diarrhea respectively. Under the same experimental conditions, loperamide (5 mg/kg) caused 99.7 ± 0.3 % reduction of diarrhea (Table 1).

Table 1: Effect of AECC and MECC on castor oil induced diarrhea in rats. Diarrhea was induced by castor oil (10 ml/kg). Values were expressed as mean ± SEM (n=6); ** significantly different from respective control ($p < 0.01$).

Treatment (mg/kg)	Osent of diarrhoea (min)	Number of rats with diarrhea	Mean weight of wet stools (g)	Mean weight of dry stools (g)	% POF	% inhibition
Distilled water (Control)	62.3 ± 1.7	6⁄6	5,7 ± 0,6	0.8 ± 02	-	-
AECC (50 mg/kg)	143.3 ± 13.1**	3⁄6	1.8 ± 0.8	0.4 ± 0,1	23.8 ± 11.6	76.3 ± 11,5
AECC (100 mg/kg	200.0 ± 0.8	2⁄6	0.7 ± 0.5	0.1 ± 0.1	38.0 ± 11.2	86,6 ± 11.1
AECC (200 mg/kg)	230.0 ± 3.3	1⁄6	0,7 ± 0,59	0,06 ± 0,05	40,87 ± 6,81	93,19 ± 6,81
MECC (50 mg/kg)	132,67 ± 2,62	3⁄6	1,67 ± 0,76	0,40 ± 0,22	20,58 ± 9,28	79,42 ± 9,28
MECC (100 mg/kg)	195 ± 8,66	2⁄6	0,66 ± 0,42	0,04 ± 0,03	12,61 ± 2,54	97,25 ± 1,89
MECC (200 mg/kg)	-	0⁄6	-	-	-	100
Loperamide (5 mg/kg)	200	1⁄6	0,61	0,01	28,99 ± 0,26	99,71 ± 0,26

Small intestinal transit

The AECC and the MECC drastically decreased propulsion of charcoal meal through the gastrointestinal tract at 50 mg/kg (35.9 ± 2.1 % and 41.5

± 2.3%); 100 mg/kg (40.8 ± 2.3% and 54.2 ± 3.4%); 200mg/kg (52.1 ± 1.3%, and 67.7 ± 1.3%), respectively in comparison to the control. The standard drug loperamide reduced up to 62.5 ± 1.4% (Table 2).

Table 2: Effect of AECC and MECC leaves on castor oil induced small intestinal transit in rats. Castor oil (10 ml/kg) was used to induce small intestine transit. Values were expressed as mean ± SEM. (n=6); **** significantly different from respective control ($p < 0.0001$).

Group	Treatment	Mean length of intestine (cm)	Mean distance travelled by charcoal (cm)	Peristaltic Index	% Inhibition
I	Distilled water (Control)	101.08 ± 1-19	81.88 ± 1.99	81.13 ± 2.60	-
II	AECC (50 mg/kg)	99,78 ± 1,45	51,67 ± 1,43	51,86 ± 1,81	35,92 ± 2,14
III	AECC (100 mg/kg)	99,63 ± 1,36	47,68 ± 1,82	47,93 ± 2,07	40,80 ± 2,30
IV	AECC (200 mg/kg)	104 ± 1,39	40,33 ± 1,15	38,74 ± 0,92	52,06 ± 1,26
V	MECC (50 mg/kg)	99,63 ± 1,36	47,78 ± 1,90	48,03 ± 2,15	41,49 ± 2,28
VI	MECC (100 mg/kg)	106 ± 0,58	39,58 ± 2,35	37,36 ± 2,29	54,22 ± 3,43
VII	MECC (200 mg/kg)	113 ± 1,65	30,1 ± 1,14	26,55 ± 1,13	67,65 ± 1,25
VIII	Loperamide (5 mg/kg)	107 ± 1,93	32,42 ± 1,27	30,28 ± 0,94	62,53 ± 1,41

Castor oil induced enter pooling

In this castor oil-induced enter pooling experiment, the administration of AECC and MECC reduced the volume of intestinal fluid and weight of the intestinal contents in rats in a dose-dependent manner. Maximum percentage inhibition of the volume of intestinal contents was observed at 200 mg/kg AECC or MECC, being 62.8 ± 2.8% for MECC and 52.3 ± 2.5% for AECC compared to normal saline control group when loperamide inhibited 61.2 ± 0.7%. The results are shown in table in Table 3.

Table 3: Effect of AECC and MECC on castor oil induced enteropooling in rats.

Group	Treatment	Mean weight of intestinal content (g)	Mean Volume of intestinal contenant (ml)	% Inhibition
I	Distilled water (Control)	3,75 ± 0,94	2,57 ± 0,21	-
II	Co +AECC (50 mg/kg)	2,58 ± 0,17	2,27 ± 0,17	45,16 ± 4,27
III	Co+ AECC (100 mg/kg)	2,07 ± 0,41	2,13 ± 0,25	34,74 ± 2,92
IV	Co+ AECC (200 mg/kg)	1,94 ± 0,29	1,90 ± 0,18	52,33 ± 2,53
V	Co+ MECC (50 mg/kg)	2,09 ± 0,09	2,15 ± 0,18	46,19 ± 5,02
VI	Co+ MECC (100 mg/kg)	1,87 ± 0,14	1,85 ± 0,15	58,00 ± 1,77
VII	Co+ MECC (200 mg/kg)	1,92 ± 0,21	1,97 ± 0,26	62,83 ± 2,78
VIII	Co+ loperamide (5 mg/kg)	1,88 ± 0,29	1,47 ± 0,14	61,19 ± 0,65

***In vitro* experiments**

Effect of HMECC, on Ach or KCl-evoked contractions in isolated jejunum of rat

Since the AECC and MECC did not present significant effects *in vivo* assays, the *in vitro* assays were carried out using the hydromethanolic extract (HMECC). HMECC cause a concentration-dependent relaxation of isolated jejunum rat tissues precontracted with Ach .The contractions induced by Ach (10 µM) or by KCl (100 mM) were significantly inhibited in rat jejunum preparations when adding the cumulative concentrations of HMECC. The Ach and the KCl-induced contractions in the jejunum were inhibited by HMECC in a concentration-dependent manner with IC50 values of 0.075 ± 0.012 3mg/ml and 0.182 ± 0.041 3mg/ml in contractions induced by Ach (10 µM) and KCl (100 mM), respectively, as shown in Figure 1.

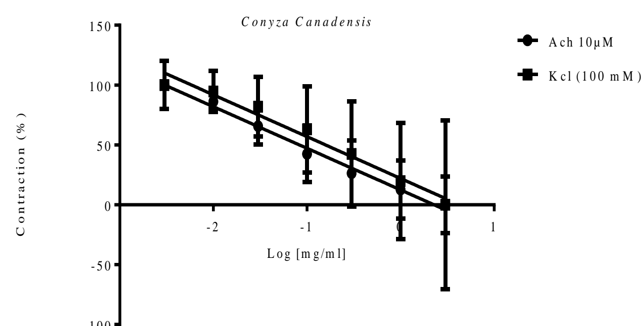

Figure 1: Spasmolytic effect of cumulative concentrations of HMECC on Ach (10 µM; fill circles) and KCl (100 mM; fill squares) induced

contractions in rats isolated jejunum. n=5.

Effects of L-NAME on the spasmolytic effect induced by HMECC on ach-precontracted rat jejunum

In order to determine whether the spasmolytic effect of HMECC implicates the NO pathway activation, rat jejunum segments were incubated for 20 min in the presence of L-NAME, a selective NO synthase inhibitor. The spasmolytic effect of HMECC was not significantly affected in the presence of L-NAME as compared with the control. The IC50 values of HMECC and L'NAME were 0.0491 ± 0.032 mg/ml and 0.126 ± 0.076 mg/ml respectively; therefore it seems that the observed spasmolytic effect of HMECC does not implicate the NO pathway activation as shown in figure 2.

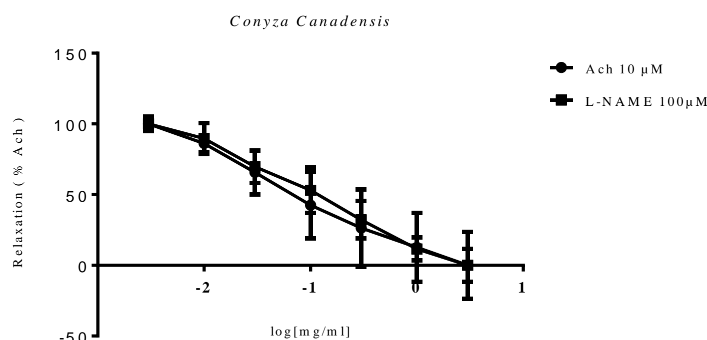

Figure 2: Spasmolytic effect of cumulative concentrations of HMECC in the presence of L-NAME (100 µM) in rat isolated jejunum (n=5).

Antioxidant activity of AECC

In the present investigation, two analytic methods namely DPPH and FRAP were used for the evaluation of antioxidant activity of C. Canadensis. Gallic acid and Butylated Hydroxytoluene (BHT) were used as the standard compound.

DPPH radical scavenging assay

The DPPH radical scavenging capacity results of AECC are shown in Figure 3 as comparable with antioxidant standards gallic acid and BHT. AECC exhibited a concentration-dependent antiradical property (IC50 = 0.44 ± 0.17 mg/ml), about three times less potent than the standards gallic acid (IC50= 0.13 ± 0.03 mg/ml) and BHT (IC50=0.17 ± 0.04 mg/ml).

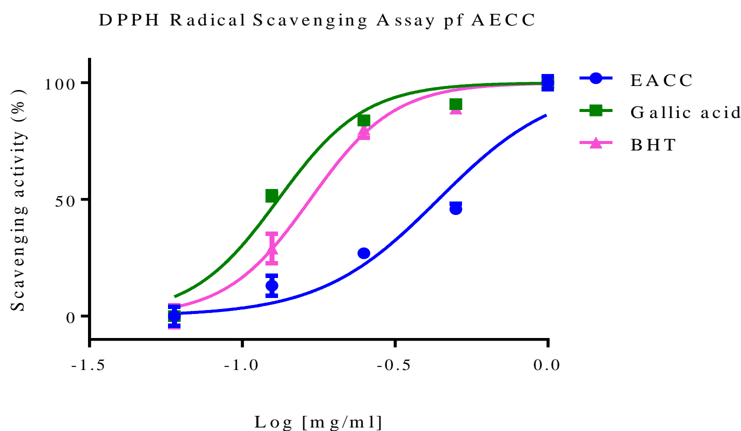

Figure 3: DPPH radical scavenging assay of EACC and standards (gallic acid and butylated hydroxytoluene).

Reducing power: FRAP

The reductive capability of the AECC compared with antioxidant standards gallic acid and BHT has been illustrated in Figure 4. AECC causes a concentration-dependent (0.06 1 mg/kg) antioxidant activity as caused by gallic acid and by BHT. The maximal antioxidant effect was reached with 1.8 ± 0.2, 2.2 ± 0.2 and 1.2 ± 0.4 mg/ml of EACC, of gallic acid and of BHT, respectively. The IC50 values were 0.25 ± 0.09, 0.56 ± 0.11 and 0.34 ± 0.22 for the extract, for gallic acid and for BHT, respectively.

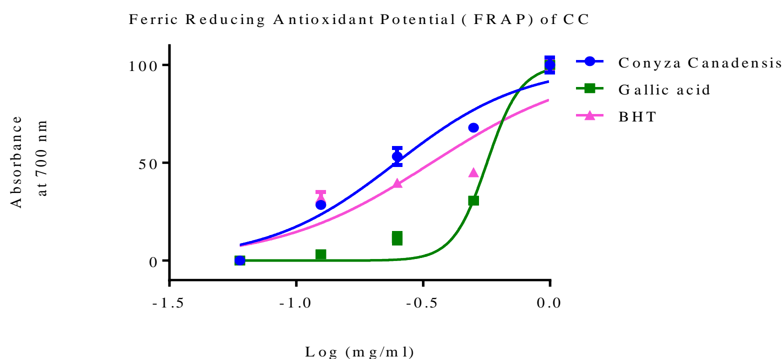

Figure 4: Total reduction capacity of standards (Gallic acid, BHT) and AECC.

Fourier Transform Infrared Spectroscopy (FTIR)

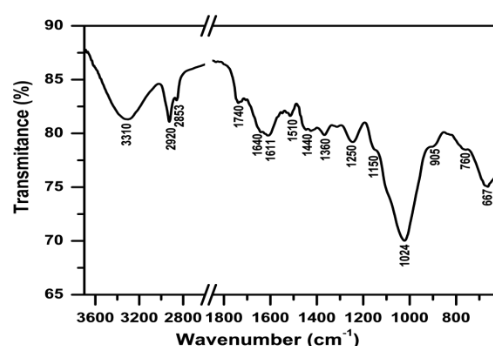

Figure 5: Fourier transform infrared spectroscopy (FTIR) spectrum of the aqueous extract of C. Canadensis extract.

As shown in Figure 4, the FTIR spectrum investigation exhibits the presence of interesting characteristic functional bonds and chemical groups constituting the structural and molecular aspects of C. Canadensis as illustrated in table 4.

Table 4: The main FTIR band assignments of C. Canadensis extract. ν: stretching; ν_{as}: asymmetric stretching; ν_s: symmetric stretching; δ_{as} asymmetric bending; δ_s symmetric bending:δ: in-plane deformation; γ: out of plane bending; δ_{oop}: out of plane bending.

Wavenumbers (cm^{-1})	Assignment and description of vibrational mode	References
3700-3100	νOH (*broad*): polyphenol + glucopyranosidic ring (*inter*- and *intra*-molecular hydrogen bonds)	[17] [18]
2920 + 2853	$\nu_{as}CH_2$ + ν_sCH_2: side chain of glucopyranosidic ring	[19][20][21]
1740	νC=O ester: conjugate carbonyl in esterifed phenolic acids (Ar-CO-O-sugar)	[22]
1640	νC=O ketone: diconjugate carbonyl (Ar-CO-O-Ar and/or Ar-CO-O-C=C)	[23] [24] [25]
1625	δO-H of adsorbed water	
1611 and 1510	$\nu C_{ar}=C_{ar}$ (*strong*): polar aromatic bonds related to phenol	[23] [17] [25]
1440	$\delta_{as}CH_2$ (*scissoring*): side chain of pyranose ring	[24] [20]
1360	δ_sCH_2 (*wagging*): side chain of glucosidic ring + νC-O-C of sugar acetal and δC-CO-O angular bending of ester group	[26]

1250	νC_{ar}-O of hydroxyl group linked to aromatic ring	[27] [25] [21]
1150	ν_{as}C-O-C: bridge of β-glycosidic linkage or νC-O-C of carbohydrates	[26] [28] [20] [21]
1024	νC-OH (very *strong*): typical glycosidic ring (fingerprint of carbohydrate)	[29] [28] [21]
905	C-H deformation of glucose characteristic of β-glycosidic bond	[30]
860	γC-H_{ar}: 1 isolated CH_{ar}, fingerprint of 1,3,4,5-tetrasubstituted hydroxyphenolic: (sinapylyl *i.e*. syringyl derivatives)	[31] [22] [21]
760	δ_{oop}C-H_{ar} :4 or 5 adjacent CH_{ar}, fingerprint of mono and/or 1,2 -disubstituted hydroxy-phenolic and/or δO-C-O of carbohydrate	[23] [29]
850-600 (centered at 667)	δ_{oop}O-H (*broad*): of carbohydrate and phenols	[20] [32]

DISCUSSION

The knowledge available on the safety, efficacy and mode of action and potential adverse reactions of C. Canadensis are far from sufficient to meet the criteria needed to support its use. Since safety continues to be a major issue with the use of herbal remedies, it becomes imperative, therefore, to perform further scientific studies to ascertain the efficiency and safety of C. Canadensis, to explore its mechanisms of action on antispasmodic and antidiarrheal properties and to provide scientific proves for the traditional utilization of this plant in the folk medicine. Hence in our present study we investigated the safety of Conyza condenses through the acute toxicity study. The results of acute toxicity demonstrated that AECC and EMCC did not cause any death or any signs, symptoms of toxicity or mortality in any of the animals tested when administering our plant extracts at the dose of 800, 2000, 3000, 5000 mg/kg. Up to 5000 mg/kg, the extracts did not cause any death or any signs, symptoms of toxicity or mortality in any of the animals tested what may correspond to 300 g for a 60 kg person, an amount incomparable higher than that normally consumed. The oral LD50 was therefore greater than 5000 mg/kg in rats, which means that C. Canadensis is safe.

The present study was designed to investigate experimentally the antidiarrheal activity of extracts of C. Canadensis. The model used was the diarrhea induced by castor oil in Wistar rats. It is well documented that castor oil produces diarrhea due to its most active metabolite, ricin oleic acid. The release of ricin oleic acid as results of lipase enzymes in the upper part of the small intestine causes , as a consequence of its binding to EP3 prostanoid receptors on smooth muscle cells, irritation and inflammation and stimulates peristaltic activity of the intestinal mucosa, leading to release of prostaglandins and nitric oxide, which stimulate motility and secretion [33,34]. Thus cause hyper secretory response and prevent the reabsorption of sodium chloride and water [35].

In the present study it is demonstrated that oral administration of extracts of C. Canadensis to the rats produced a significant effect on all parameters measured: onset of diarrhoea, the frequency of defecation, fecal dropping and the mean weight of feces. Castor oil is also reported to induce diarrhea through the activation of Cl- channels, favoring Cl- efflux from the cell which results in massive secretion of water into the intestinal lumen and profuse watery diarrhea [36]. Our extracts considerably increased the reabsorption of sodium chloride and water by decreasing intestinal motility since it decreased the intestinal transit by charcoal meal. It is worth mentioning here that the antidiarrheal effect of C. Canadensis extracts was greater than loperamide which was described to regulating the gastrointestinal tract, to reduce transit in the intestine, and to decrease colon flow rate, and consequently any effect on colonic motility [37]. It's well reported that the therapeutic effect of lope amide is to be due to its antimotility and antisecretory properties [38].Taking all together, we think the mechanism by which the EACC and MECC may promote their antidiarrheal effect may be due to decrease of the intestinal motility and the inhibition of the release of endogenous prostaglandin or alteration of the activity of Na+K+ATPase or activation of chloride channels.

The spasmolytic effect of HMECC was evaluated using several spasm genic agents including Ach (10 µM), KCl (100 mM), L-NAME (100 µM), in order to determine the mechanism behind the spasmolytic effect of HMECC. The results obtained indicated that the spasmolytic effect of HMECC on contractions induced by Ach (10 µM) and KCl (100 mM) was concentration-dependent and reversible after washing, suggesting that this inhibition was not due to damage of the intestine by the extract.

Different mechanisms are involved in gastrointestinal smooth muscle relaxation. These include, blockade of calcium voltage channel, the activation of the NO pathway, the implication of muscarinic receptors therefore, in this study we explored these mechanisms in the studied spasmolytic effects of HMECC.

It is well documented that the [Ca2+], is responsible for regulator of smooth muscle contraction of smooth muscle preparations are dependent upon an increase in the cytoplasmic free [Ca2+], which activates the contractile elements [39]. The increase in intracellular Ca2+ is due to either influx *via* voltage dependent Ca2+ channels (VDCs) or to release from intra- cellular stores in the sarcoplasmic reticulum.

It's well known also that the substances which inhibit the contractions promoted by high K+ are considered to be a calcium voltage channel blocker. Since, HMECC inhibits the contraction of rat jejunum preparation induced by high K+ we assume that the spasmolytic effect acts *via* the

restriction of Ca2+entry *via* voltage dependent channels. Regarding the fact that HMECC successfully inhibited the contractions provoked by Ach and KCl in concentration-dependently, we think that it is likely that the extract contains compounds that interfere with calcium channel activity or with release of calcium ions from intracellular stores.

Furthermore, numerous studies have clearly demonstrated the key role of the nitric oxide in mediating noradrenergic–noncholinergic smooth muscle relaxation through the gastrointestinal tract [40]. Endogenous NO from the smooth muscle plays an important role in the physiologic regulation of vascular tone. NO acts *via* an activation of soluble guanylyl cyclase (sGC) which increases the production of intracellular cyclic guanosine monophosphate (cGMP). The increase of cGMP results in the activation of protein kinase G that decreases calcium influx probably by increasing uptake of Ca2+ through activation of sarcoplasmic reticulum Ca2+ - ATPase (SERCA) [41]. In order to explore whether the antispasmodic property displayed by HMECC was mediated by the NO pathway activation, the effect of HMECC on pretreatment with L-NAME (100 µM) an inhibitor of Nitric Oxide Synthase (NOS) was tested .The results have shown that the relaxation curves of HMECC was not modified by the presence of L-NAME and was similar to the control and indicating hence that the relaxation exhibited by HMECC was not due to the nitric oxide signaling pathway.

The antioxidant data showed remarkable antioxidant capacity of AECC. The antioxidant potential of AECC stronger than the positive controls gallic acid and BHT these results show that AECC exhibited significant antioxidant activity. The antioxidant activity of AECC was also assessed using FRAP and our findings displayed that AECC possesses antioxidant activity in a concentration-dependent manner. Considering the concentration 1 mg/ml extract/standard the reducing power activity of AECC, gallic acid and BHT. Our experimental data revealed that the aqueous extract of C. Canadensis has an important antioxidant activity and in a concentration dependent manner in comparison with gallic acid and BHT, thence, it seems that extracts of C. Canadensis possesses the ability to protect the body from damage caused by free radical induced by oxidative stress.

Fourier transform infrared spectroscopy was carried out in C. Canadensis extract in order to determine the presence of functional groups and elucidate the chemical structure. The advantage of this technique is non-destructive, preserves the integrity of the sample and enables the identification of each signal.

The FTIR profile spectrum of C. Canadensis extract (Fig 5) is characterized by three main spectral regions: 3650-3100, 3000-2850 and 1800-600 cm^{-1}. Basing on previous literature data, the later interval is with great interest for structural characterization exhibiting three fingerprint spectral and can in turn be subdivided into three more informative sub-regions: 1800-1630, 1620-1200 and 1200-900 cm^{-1} showing a single and specific fingerprint to each area. The summary of FTIR attributions of C. Canadensis extract was done in accordance with the literature data and can be found in Table 4.

In the range of 1800-1630 cm^{-1}, the band at 1740 cm^{-1} is clearly distinguishable and characterizes the stretching vibration of conjugate carbonyl in esterified phenolic acids (Ar-CO-O-sugar), the relative weak intensity of this band could be connected to low content of ester group (-CO2R) [31,26]. Additionally, the strong signal at 1640 cm^{-1} is in favor of diconjugated aromatic carbonyl in ketonic form (Ar-CO-O-Ar), this profiling might be due to the superimposition of adsorbed water absorbing generally at 1620 cm^{-1} [23,42,20,29].

Concerning the sub-region of 1620-1200 cm^{-1}, the strong signal at 1611 cm^{-1} depicting the presence of polar aromatic Car=Car in phenol compounds (antioxidants) with the contribution of the other weak Car=Car band at 1510 cm^{-1} [22,42,25] .The weak and large absorption signal at 1250 cm^{-1} is due to polar character of the stretching mode of Car-O linked to OH in phenolic compounds [31,25]. The two appearing shoulders at 860 and 760 cm^{-1} are with great importance (fingerprint) and affected to out of plane bending C-Har aromatic. The latest peaks inform on the degree of substitution in phenolic rings [21]: 1,3,4,5-tetrasubstituted and mono or bi-substituted positions (1 and 2), respectively, suggesting thus the presence of the following nuclei, sinapylyl nucleus (syringyl derivatives) in the first case and benzoyl or cinnamyl in the second case. The both phenolic and carbohydrates compounds were confirmed by their broad absorption bands centered at 3310 cm^{-1} in the range of 3650-3100 cm^{-1}, ascribable to O-H stretching vibration.

As for the area of 1200-900 cm^{-1}, the most intense and strong absorption band centered at 1024 cm^{-1} involves the C-O fingerprint stretching vibration well recognizable in the carbohydrates ring (glucopyranose) [26,20,21]. The overlapped peak in the form of shoulder at 1150 cm^{-1} is related to C-O-C bridge of β-glycosidic linkage or C-O-C of carbohydrates (glucose). Others primary and secondary C-O alcohols bands of sugar ring were hidden by the intense peak at 1024 cm^{-1}. The broad signal but less intense centered at 667 cm^{-1} is connected to the out of plane bending mode of O-H in carbohydrates (glucose). Concerning the peak at 905 cm^{-1}, it corresponds to C-H deformation mode of glucose which is characteristic of β-glycoside bond in carbohydrates [30].

Furthermore, the two arising peaks at 2920 cm^{-1} (asymmetric stretch) and 2853 cm^{-1} (symmetric stretch) are related to CH2 in side chain of glucopyranose ring, supported by both in plane bending vibrations: scissoring (asymmetric) at 1440 cm^{-1} and wagging (symmetric) at 1360 cm^{-1} [21,19]. While the intracyclic tertiary C-H peaks of glucosidic ring were overlapped by the stretching vibration of CH2 signal base. The broadening in the 1360 cm-1 peak could be due to the contribution of other bonds, as O-C-O acetal related to sugar and angular bending vibration of C-(C=O)-O lied to ester group.

Thus, from these findings, it is concluded that the main structural Conyza canadenis extract constituents are, the richness in polar phenolic compounds (antioxidants) in both forms esterified and ketonic. The acidic and mainly phenolic OH contribute to the increase in polarity and serve as an intermediary for connecting sugar molecules, resulting the formation of new former, such as esterified phenolic acid or asymmetric ether groups.

CONCLUSION

Given the current evidence, AECC, HMECC and MECC may be considered as a potential therapeutic option in the prevention and the treatment

of gastrointestinal heath challenges along with their ability to protect the body from oxidative stress and have provided data on their mechanisms of action. The current research work might be useful to identify new molecules with potential effect on gastrointestinal health challenges, which can also turn out to be good therapeutic approaches for gastrointestinal diseases and the oxidative stress. However further studies are needed in order to identify the main compound(s) responsible (s) for the studies pharmacological properties and our coming research will reveal indeed these compounds.

DECLARATION OF COMPETING INTERESTS

Authors have declared that no competing interests exist

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