Available online on 15.02.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Evaluation of phytochemical, antioxidant, and *In-vitro* antidiarrhoeal, activity of *Euphorbia hirta*

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ABSTRACT

Background: The current study was carried out to evaluate the phytochemical, antioxidant, and *In-vitro* antidiarrheal properties of *Euphorbia hirta*

Methods: Extracts were obtained using cold extraction, hot extraction and autoclave extraction methods using Methanol, Chloroform, Petroleum ether and distilled water as solvents. Crude extracts were screened for different phytochemical constituents like sugars, saponins, flavonoids, tannins, and glycosides etc. Antioxidant activity was evaluated using spectrophotometric method. The *in-vitro* antidiarrheal activity was elucidated by the antimicrobial activity using agar diffusion method.

Results: Methanol proved to be a good solvent for extraction. *In-vitro* antidiarrheal activity was shown by all extracts on *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi* bacteria which are mainly responsible for diarrhea.

Conclusion: From the phytochemical screening it shows presence of phytochemicals like alkaloids, tannins, glycosides, and saponins. The plant also shows antioxidant activity, methnolic extracts shows higher activity and it shows *In-vitro* antidiarrhoeal activity which clearly indicates that the plant can be used for the treatment of diarrhea. Further studies should be done to isolate the compound responsible for activity in the experimental animals.

Keywords: *Euphrobia hirta,* autoclave extraction, antidiarrheal, phytochemicals.

Article Info: Received 05 Jan 2019; Review Completed 09 Feb 2019; Accepted 11 Feb 2019; Available online 15 Feb 2019

Cite this article as:

Bhat SA, Mir SA, Maqbool M, Bhat AU, Masoodi MH, Evaluation of phytochemical, antioxidant, and *In-vitro* antidiarrhoeal, activity of *Euphorbia hirta*, Journal of Drug Delivery and Therapeutics. 2019; 9(1-s):290-294

DOI: http://dx.doi.org/10.22270/jddt.v9i1-s.2352

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INTRODUCTION

Diarrhea is passing looser or more frequent stools than in normal and it affects most people from time to time and is not worried[1]. However, it can be distressing and unpleasant until it passes, which normally takes a few days to a week. There are many different causes of diarrhea, but a bowel infection is a common cause in both adults and children. Infection may be caused by: Virus such as norovirus or rotavirus. Bacteria such as *Escherichia coli, salmonella typhi*, and *campylobacter staphylococcus aureus* which are spread in contaminated food. Parasite – such as the parasite that causes giardiasis, which is spread by contaminated water these infections may be coughed during travel abroad, particularly in areas with poor standards of public hygiene known as traveler's diarrhea[2]. Diarrhea can also be the result of anxiety, a food allergy, medication, because of long-term condition, such as irritable bowel syndrome but these type of diarrhea clear up after a few days without treatment, and one should drink plenty of fluids as diarrhea leads to dehydration. According to WHO, Infectious diarrhea is one of the most common diseases in the world it is one of the five most important causes of death. Recent data from Germany and the USA indicate that in developed countries approximately one case of acute gastroenteritis occurs per person per year in adults[1].

MATERIALS AND METHODS

Extraction process for *Euphorbia Hirta*

Three methods are used for extraction Cold extraction, hot extraction, and autoclave extraction[3].

Cold extraction

In cold extraction 50g of dry powder was weighed and to it 250ml of water was added in 500ml beaker. The extract was shaked in rotary shaker for 24 hours after this filtration was done and also centrifugation was carried out for 10 mins at the rate of 4500 rpm. The extract was concentrated on water bath and dried. Percentage yield was 18%.

Hot extraction

In hot extraction 50g powder drug was weighed and transferred in 500ml beaker to this 250ml water was added. The extract was heated in water-bath for two hours at 80°C. After two hours, the extract was filtrated and then

centrifugation was done for 10 mins at rpm 4500. The extract was concentrated in water-bath at 100°C and dried the %yield was 25%.The powder was dried overnight then soxhlet apparatus was used for extraction 40g of powder which was previously extracted with water are loaded in soxhlet apparatus to this 250ml methanol was added and soxhlet runs at 30°c until six refluxes occurs and clear methanol is shown in Safin tube. The extract was concentrated on water bath and dried. The similar process was run for chloroform also for petroleum ether the % yield for methanol was 6.25% for chloroform 2.5% and for petroleum ether 1%[4].

Autoclave extraction

In this method dry powder of 50g was weighed and transferred in a 500ml conical flask to this 250ml water was added to the flask. Shaked and autoclaved it for 15 mins at temperature 121°c and pressure 15lbs. After 15 mins the sample was cooled at room temperature and filtered through muslin cloth. The obtained filtrate was subjected to centrifugation at rpm 4500 for 10 mins at room temperature. The extract was concentrated on a heating filament at 25°c. The concentrated extract was transferred to petri plates and kept on water bath at 80°c for further evaporation of the solvent. After one hour, dried extract has been obtained and the percentage yield was 18%.

Phytochemical Screening

Phytochemical analysis of extract was done to identify the various phytoconstituents like alkaloids, steroids, saponins, sugar, flavonoids, tannins, and glycosides and mucilage's. The concentration of the aqueous extract was kept 1mg/ml for this study[5].

Test for sugar (Molisch's test)

1 ml of test sample was taken in a test tube and mixed with 2 drops of Molisch's reagent. To this solution, 1 ml of concentrated sulfuric acid was added. Molisch's reagent was prepared by dissolving 10 g of α-Napthol in 100 ml of s added from side of the inclined test tube, so that 2 the acid formed a layer beneath aqueous solution without mixing with it. A red brown ring appears at the common surface of the liquids indicating presence of sugar.

Test for saponins (Foam test)

The sample (1mg/1 ml) was taken in a test tube and diluted with 2 ml of distilled water. It was shaken by hand for 15 min. A foam layer was obtained at the top of the test tube. This foam layer indicates presence of saponins. Test for steroids Crude plant extracts (1 mg/ml) was taken in a test tube, dissolved with chloroform (10 mL) and an equal volume of concentrated sulfuric acid was added to the test tube by sides. The upper layer in the test tube turns into red and sulfuric acid layer showed yellow with green fluorescence which indicates presence of steroids.

Test for flavonoids

One ml sample (1mg/1 ml) was taken in a test tube and added a few drops of 10% NaOH solution. An intense yellow color appears in the test tube. After addition of a few drops of dilute acid, it became colorless that indicates presence of flavonoids.

Test for tannins

One ml of aqueous extract (1mg/1 ml) was added to 1 ml of distilled water and a few drops of 5% ferric chloride (dissolved in 90 % ethyl alcohol) solution were added. A dark green or blue green color formed, which showed presence of tannins.

Test for glycosides

Crude plant extracts (1 mg/ml) was taken in a test tube and added few drops of Molisch's reagent. Mixed it and add 2ml of concentrated sulfuric acid carefully through the side of test tube. Formation of reddish violet ring indicates presence of glycosides.

Test for alkaloids (Mayer's reagent)

Mayer's reagent was prepared as follows: 1.36 g of mercuric chloride and 5g potassium iodide dissolved in 100 ml of distilled water. 1 ml plant extract (1mg/1 ml) was taken in a test tube and 3 a few drops of Mayer's reagent was added. Cream color precipitates out, which indicates presence of alkaloid.

Determination of Total Phenolic Content

Phenolic contents of all extracts prepared in DMSO at 10mg/ml were estimated, using method of Taga[6]. Briefly, 100 µl aliquots of sample were mixed with 2.0 ml of 2% Na2CO3 and allowed to stand for 2 min at room temperature (RT). After incubation, 100 µl of 50% **Folin–Ciocalteu's** phenol reagent was added, mixed thoroughly and allowed to stand for 30 min at RT in dark conditions. Absorbance of all the solutions were measured at 720 nm using GENESYS 10 UV Spectrophotometers (Spectronic Unicam). Phenolic contents were expressed as Gallic acid equivalent per gram (GE/g).

Evaluation Antioxidant Activity

Total antioxidant activities of extracts (10 mg/ml of DMSO) were determined using spectrophotometric method [7]. Briefly, 0.3 ml of sample was mixed with 3.0 ml reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min in a water bath. Absorbance of samples mixture were measured at 695 nm using GENESYS 10 UV Spectrophotometers (Spectronic Unicam). Total antioxidant activity was expressed as number of equivalents of ascorbic acid.

In-Vitro Antidiarrheal Activity

Three bacterial strains were used which are mainly responsible for diarrhea *Escherichia coli* (MTCC443) *Salmonella typhi* (MTCC1688) *Staphylococcus aureus* (MTCC96)[8]

The antibacterial screening was carried out using the agar diffusion method as described by[9] with slight modifications. The standardized inoculum (100µl) of (0.5 Mc Farland) of each test bacterium was inoculated on the plates and spread with the help of spreader. Plant extracts at the concentration of (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) were loaded into different peripheral wells. Gentamycin (10µg/disc) disc was placed in each Petri plate which served as positive control, while as 10% Dimethyl sulfoxide served as negative control in a separate Petri plate. The Petri plates were incubated at 37°C for 18-24 hours in an incubator. The plates were then observed for the zones of inhibition. Antibacterial potential was evaluated by measuring the diameters of zones of inhibition in millimeters (mm) with the help of a standard measuring scale.

RESULTS

Table 1: Phytochemical screening of *Euphorbia hirta*

Sr. No.	Variable	Methanol	Chloroform	Petroleum Ether	HWE	CWE	Autoclave
1.	Sugar	+	+	+	+	+	+
2.	Saponins	-	+	+	+	+	+
3.	Steroids	+	-	-	+	+	+
4.	Flavonoids	+	+	+	+	-	+
5.	Tannins	+	+	+	+	-	+
6.	Glycosides	-	+	+	+	+	+
7.	Alkaloids	+	-	-	+	+	+

(+) = Presence, (-) = Absence

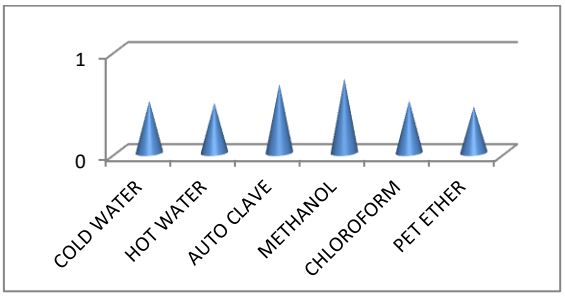

Figure1: Total phenolic content (Abs.721nm)

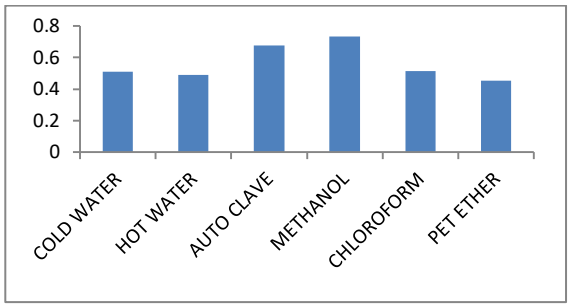

Figure 2: Antioxidant activity (Abs.695nm)

Table 2: *In-vitro* antidiarrheal activity

Strain	Zone of inhibition (mm)					
	Hot 20mg/ml	Cold 20mg/ml	Methanol 20mg/ml	Chloroform 20mg/ml	Petroleum ether 20mg/ml	Autoclave 20mg/ml
Escherichia coli (MTCC443)	12	14	17	18	_	13
Staphylococcus aureus (MTCC96)	16	14	18	14	15	_
Salmonella typhi (MTCC1688)	12	_	_	_	_	13

(-)= no activity (mm) = millimeter

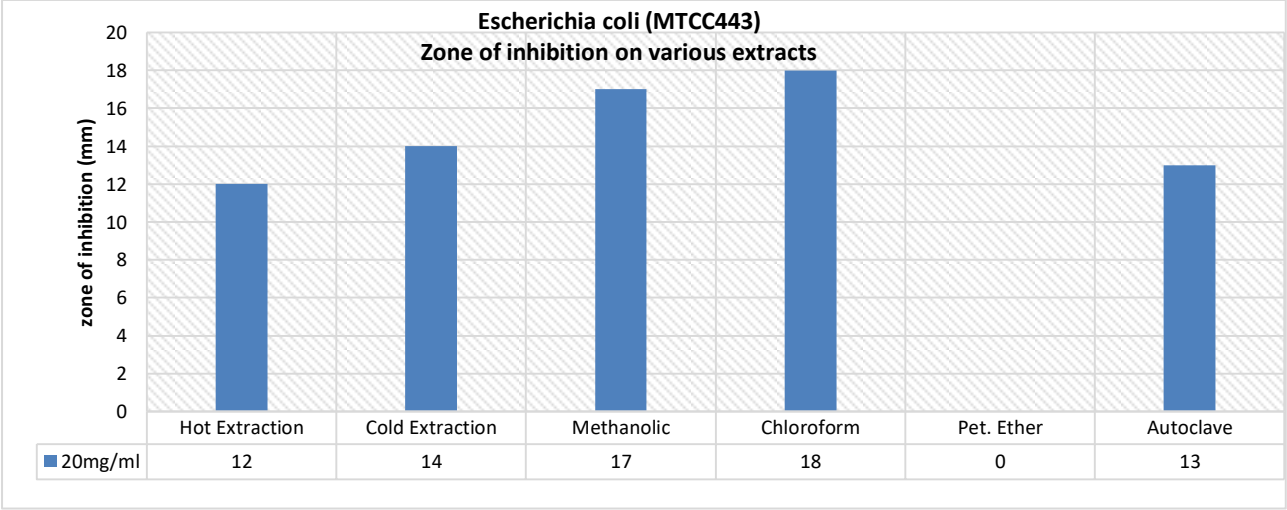

Figure 3: *In-vitro* antidiarrheal activity of *Escherichia coli* (MTCC443)

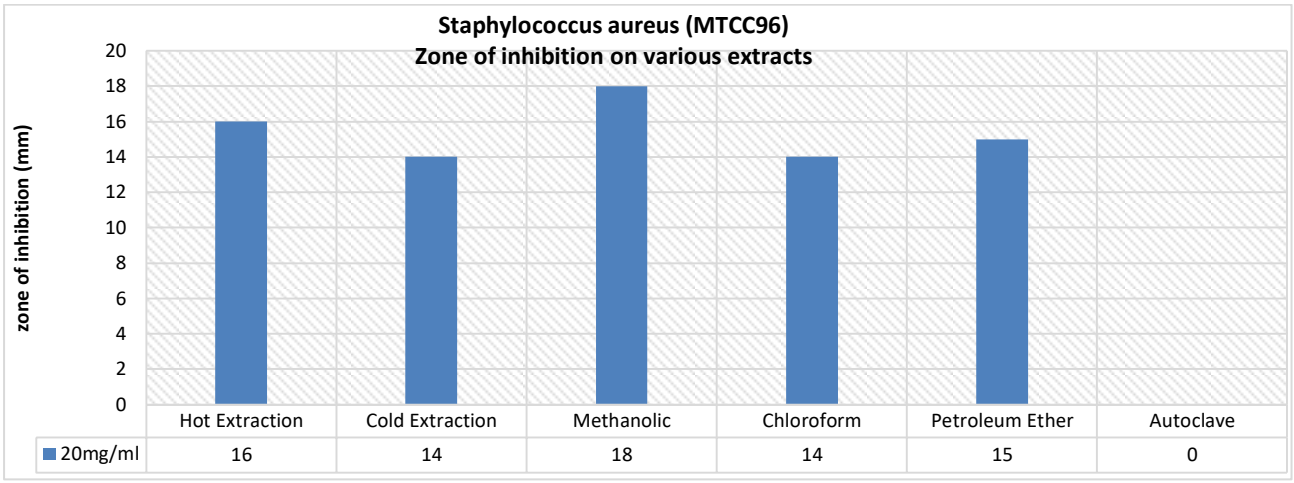

Figure 4: *In-vitro* antidiarrheal activity of *Staphylococcus aureus (* MTCC96)

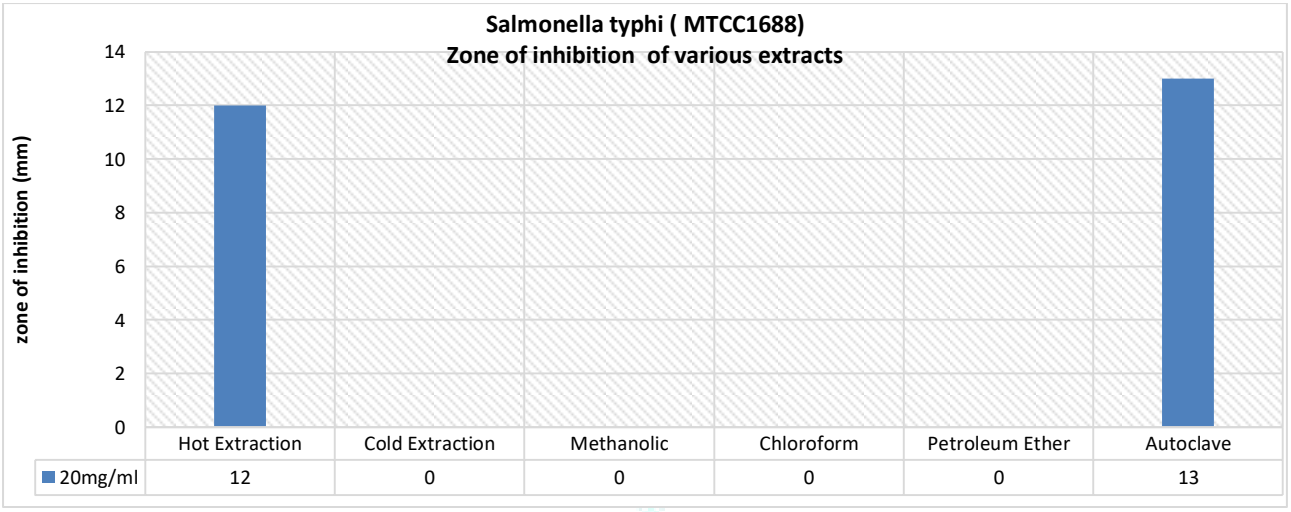

Figure 5: *In-vitro* antidiarrheal activity of *Salmonella typhi(* MTCC1688)

DISCUSSION

The plant *Euphorbia hirta* was traditionally used to treat diarrhea. The aim of this study was to evaluate the antidiarrheal activity by *in-vitro* method. In the extraction method the hot extraction, cold extraction and autoclave extraction methods were used. For the hot extraction soxhlet apparatus was used and solvent used where petroleum ether, chloroform, methanol, and water but against the polarity. In cold extraction solvent used was water and in autoclave extraction method solvent used was water. Phytochemical screening of these extracts shows presence of alkaloids, tannins, saponins, glycosides and sugars. *In-vitro* antidiarrhoeal activity was done by agar diffusion method all the extracts have shown activity but methanol shows higher activity than the rest one. Also autoclave extracts have shown activity which clears indicates the autoclave can be used for extraction.

CONCLUSION

From the phytochemical screening of the plant, it showed presence of phytochemicals like alkaloids, tannins, glycosides, and saponins. The plant also shows antioxidant activity methnolic extracts shows higher activity and it showed *In-vitro* antidiarrheal activity which clearly indicates that the plant can be used for the treatment of diarrhea. Further studies should be done to isolate the compound responsible for activity.

CONFLICT OF INTEREST: None

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