



# Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: [www.ajrcps.com](http://www.ajrcps.com)



## EVALUATION OF ANTI-DIARRHEAL ACTIVITY OF THE EXTRACTS OF CASTANEA SATIVA SEEDS

Anandarajagopal Kalusalinagm<sup>\*1</sup>, Nurul Syakirin Aina Binti Mohd Zailani<sup>1</sup>, Abdullah Khan<sup>1</sup>,  
Venkatesan Narayanan<sup>2</sup>

<sup>1</sup>School of Pharmacy, KPJ Healthcare University College, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.

<sup>2</sup>Arulmigu Kalasalingam College of Pharmacy, Krishankoil 626 190, Tamil Nadu, India.

### ABSTRACT

Diarrhea is one of the primary causes of morbidity and mortality on a global scale, leading to one billion disease episodes and approximately two million deaths each year among children under five years of age. Due to limitations associated with various treatments available, the need for developing newer drugs is imperative. The present study was aimed to evaluate the anti-diarrheal activity of the extracts of *Castanea sativa* seeds. The water and ethanolic extracts of *C. sativa* seeds were obtained by cold maceration method. Both extracts were subjected to preliminary phytochemical screening and showed the presence of alkaloids, sterols, proteins, phenolic compounds and tannins, terpenoids, carbohydrates, flavonoids, lipids and fatty acids. The anti-diarrheal activity of water and ethanol extracts was evaluated at doses of 200 and 400 mg/kg, body weight against castor oil induced diarrhea in rats. The results were statistically analyzed and compared with standard drug, Loperamide (2mg/kg). The results revealed that both extracts at different doses produced significant anti-diarrheal effect. Water extract of *C. sativa* seeds at a dose of 400mg/kg showed the best anti-diarrheal activity among all the extracts. The anti-diarrheal activity of water and ethanolic extract of *Castanea sativa* seeds might be due to the presence of pharmacologically active substance such as flavonoids, phenolic compounds and tannins.

### KEYWORDS

*Castanea sativa*, Antidiarrheal, Water and Ethanolic extracts and Castor oil induced.

### Author for Correspondence:

Anandarajagopal Kalusalinagm,  
School of Pharmacy,  
KPJ Healthcare University College,  
Kota Seriemas, Nilai, Negeri Sembilan, Malaysia.

**Email:** [anandkarg@gmail.com](mailto:anandkarg@gmail.com)

### INTRODUCTON

Diarrhea is described as an expansion in the fluidity, volume, recurrence of solid discharges, increase frequency of bowel discharge, wet feces and stomach torment, joined by increased emission and diminished absorption of liquid, and along these lines loss of water and electrolytes<sup>1</sup>. Diarrhea can be caused by infectious agents, certain drugs, plant and insect poisons, gastrointestinal disease, and any

materials that enhance gastrointestinal tract discharges. It can also be caused by either digestion of poorly absorbable foods or inflammation in the gastrointestinal tract<sup>2</sup>. Even though diarrhea is a preventable disease, it remains the second driving reason for death (after pneumonia) among kids aged under five years around the world<sup>3</sup>. The worldwide burden of diarrheal frequency and seriousness of the disease is at peak in Southeast Asian and African regions<sup>4</sup>. Poverty, floods, contaminated water supplies, children underweight, water and sanitation, lack of vitamin A, and zinc insufficiency etc., add to its frequency<sup>3,5</sup>.

According to World Health Organization (WHO) over 80% of the total population depends on traditional treatment for their essential healthcare<sup>6</sup>. There have been various reports of the utilization of traditional plants for the treatment of diarrhea disease. The primary chemical constituents in plants assumed to be responsible for anti-diarrheal action are tannins and tannic acid, flavonoids, alkaloids, sesquiterpenes, diterpenes, terpenes and terpenoids<sup>7</sup>. The anti-inflammatory property of tannins help control all signs of gastritis, esophagitis, enteritis, and irritating bowel disorder. Diarrhea is likewise treated with a successful astringent drug that does not stop the stream of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine<sup>8</sup>.

*Castanea sativa* (Sweet chest nut) is one of the blossoming plant in the family, Fagaceae and recognized as a tannin rich organic product<sup>9</sup> and one of the astringent plants. The tannins are present in the bark, wood, leaves, and seed husks of *C. sativa*. It is a native of Southern Europe, Asia and North Africa<sup>10</sup>. The decoction of its leaves has been generally utilized for treating different respiratory infections, such as asthma, cough, cold, bronchitis, expectorating, and bronchial affections. The bark extract of *Castanea sativa* has imperative anti-oxidant and cytoprotective effects and also ready to regulate some vital heart functions<sup>11</sup>. Phenolic extracts of *Castanea sativa* also showed high antioxidant capacity<sup>12</sup>. As, there is no scientific report available for the anti-diarrheal activity of the extracts of *C. sativa* seeds, the present work was

aimed to evaluate the anti-diarrheal activity of the extracts of *Castanea sativa* seeds.

## MATERIAL AND METHODS

### Plant Material

The seeds of *Castanea sativa* were collected from Genting Highland, Pahang in March 2017. The fruits were identified and authenticated by a Pharmacognosist, KPJ Healthcare University College, Nilai. A voucher specimen was deposited at herbarium KPJ Healthcare University College, Nilai. (KPJUC/CRI/PA/2017(31)).

### Preparation of extracts

The pulp of *Castanea sativa* was removed and nuts were taken out, washed and dried using hot air oven at 45-50°C. The seeds were then pulverized into coarse powder using mechanical blender and divided equally into two portions. Coarsely powdered *C. sativa* seeds (100gm) was separately macerated with distilled water and ethanol (400ml) for 6 days by cold maceration method. The water extract and ethanolic extract of *C. sativa* seeds were obtained by filtration and concentrated using rotary vacuum evaporator under reduced pressure<sup>13</sup>. Both extracts were preserved in refrigerator until further use.

### Preliminary Phytochemical Screening

Qualitative phytochemical screening for water and ethanolic extracts of *C. sativa* seeds had been carried out using standard procedure to identify the presence of various phytoconstituents in the extracts<sup>14</sup>. Test for alkaloids, carbohydrates, proteins, phenolic compounds, tannins, flavonoids, terpenoids, sterols and lipids and fatty acids were performed and the results were recorded.

### Experimental Animals

Wistar albino rats of either sex weighing about 150-250gm were acquired from KPJUC vivarium, KPJ Healthcare University College, Nilai, Negeri Sembilan and used to evaluate anti-diarrheal activity of *C. sativa* seeds on castor oil induced diarrheal animal model. The animals were kept in well ventilated room at 27°C ± 2°C, 12 h light/12 h dark cycle and 35-60% relative humidity at the animal house with free access to commercial rodent pellet diet and distilled water. This study was

approved by Institutional Animal Ethics Committee (IAEC), KPJ Healthcare University College, Nilai, Negeri Sembilan (Reference No: KPJUC/RIC/BPS/EC/2017/31). All the experiments were performed in accordance with the Code of Practice for the Care and Use of Animal for Scientific Purpose.

#### **Evaluation of Anti-Diarrheal Activity**

The anti-diarrheal activity of the water and ethanolic extracts of *C. sativa* was assessed by castor oil-induced diarrheal animal model<sup>15,16</sup>. The animals were divided into six groups of four animals each. Group I received 1ml of castor oil and served as control group. Group II received standard drug, (Loperamide, 2 mg/kg) and serve as standard group. Groups III and IV received water extract of *C.sativa* seeds (200 and 400mg/kg respectively). Groups V and VI received ethanolic extract of *C.sativa* seeds (200 and 400mg/kg respectively). Diarrhea was induced in all the overnight fasted animals by administering 1 ml of castor oil orally. The test extracts and the standard drug was administered one hour prior to the oral administration of castor oil. Each rat was kept separately and observed for diarrheal episode, for a period of 5 h. During that period, number and weight of diarrheal feces were taken and noted at every 1 h. The mean diarrhea episodes and percent protection were calculated. The anti-diarrheal activity was determined in terms of percentage inhibition at 5<sup>th</sup>h.

The percentage inhibition was calculated as follow:

$$\% \text{ inhibition} = C - T / C \times 100$$

Where, C is the weight of feces obtained in control group and T is the weight of feces obtained in test group.

#### **Statistical Analysis**

The data was expressed as Mean  $\pm$  SEM. All the results were statistically analyzed and tabulated. The statistical significance was determined using one-way ANOVA.  $p < 0.05$  was considered as statistically significant<sup>16</sup>.

## **RESULTS AND DISCUSSION**

### **Colour, Consistency and Percentage Yield of Extracts**

As illustrated in the Table No.1, the water extract of *C. sativa* was semi solid, dark brown in colour, and percentage yield of the extract was 17.8%. Whereas the ethanolic extract of *C. sativa* was mucilaginous, greenish yellow in colour and the percentage yield was 3.15%. Among the two extracts, the water extract showed the highest % yield (17.8%) compared to ethanolic extract (3.25%).

### **Preliminary Phytochemical Screening**

The preliminary phytochemical screening of extracts of *C. sativa* was carried out and the results are presented in Table No.2. The results reveal the presence of alkaloids, carbohydrates, proteins, phenolic compound, tannins, flavonoids, terpenoids, sterols, lipid and fatty acids in both the extracts.

### **Anti-diarrheal activity of *C. sativa* seeds extracts on castor oil induced diarrheal model**

From the data presented in Table No.3, it is evident that the water extract of *C. sativa* significantly decreased the frequency of stools over time in a dose-dependent manner. Result tabulated in the Table No.3 shows that the stool was decreasing over the 5 h of the experiment. The percentage of diarrheal inhibition compared to control was 67.76% and 81.97% at the doses of 200mg/kg and 400mg/kg, respectively. In addition, the water extract at dose 400mg/kg produced the highest percentage of diarrheal inhibition (81.97%) compared to the standard drug, Loperamide which is 61.74%. The ethanolic extract of *C. sativa* also significantly decreased the diarrheal feces at a dose of 200mg/kg and 400mg/kg and the percentage diarrheal inhibition was 70.49% and 71.58%, respectively.

From the results, it is observed that the weight of feces for control group was at the highest frequency compared to the other group of rats. For the first hour, water extract of *C. sativa* produced the fast effect on the inhibition of the Feces. As the time passed, the weight of feces collected was decreasing in all the animals in a dose-dependent manner. The most effective dose was water extract 400mg/kg as it produced the lowest weight of stool which is

0.33g at 5<sup>th</sup>h, compared to standard drug, Loperamide yield the weight of feces, 0.7g.

Castor oil is a good source to induce diarrhea<sup>17</sup>. 90% of ricinoleate present in castor oil is mainly responsible for diarrhea production<sup>18</sup>. Ricinoleic acid is responsible for inducing diarrhea<sup>19</sup>. Ricinoleic acid cause the prostaglandin to release and eventually enhance gastrointestinal motility and secretion of electrolytes and water which may produce local irritation and inflammation<sup>20</sup>. Furthermore, ricinoleate salts with sodium and potassium is formed in the lumen. This formation may inhibit the sodium-potassium ATPase and also may rise the intestinal epithelium permeability. This situation produce cytotoxic effect in the absorptive cells of intestine<sup>19</sup>.

The antidiarrheal activity of water and ethanolic extracts of *Castanea sativa* seeds on castor oil induced diarrheal animal models was studied. Both extracts of the seeds of *C. sativa* reduced the severity of diarrhea. Dose-dependent inhibition was observed in all diarrhea parameters such as weight of feces, total number of feces and onset of diarrhea as compared to the control group.

The anti-diarrheal properties of the medicinal plants might be due to the presence of bioactive agents such as alkaloids, flavonoids, steroids, tannins, saponins, terpenoids and phenolic compound<sup>21</sup>. The phytochemical screening of both extracts of *C. sativa* seeds revealed the presence of alkaloids, tannins, sterols, carbohydrates, protein, terpenoids, phenolic compounds and flavonoids which might be the responsible for the anti-diarrheal property of the *C. sativa* seeds.

Research on the extracts of various plant species showed that the phytochemicals such as tannins and flavonoids may enhance the colonic water and electrolytes reabsorption. Other phytoconstituents such as alkaloids and phenolic compounds also inhibit the intestinal motility<sup>22</sup>. Tannins found to be

astringent as it is a bitter plant polyphenols which either bind or shrink proteins. Tannins astringency may cause dried feeling in mouth following the intake of wine, tea or unripen fruit<sup>8</sup>. Therefore, tannins are having astringent property and could treat diarrhea. The protein precipitation caused by tannins covers the surface of the cell or tissue, acting as a barrier between the irritation and the tissue which protected from damage. This process could decrease intestinal mucous layer secretions<sup>23</sup>. Many earlier studies on plants such as *Pterocarpus erinaceus*<sup>24</sup>, *Trichiliaemetica* Vahl<sup>25</sup>, *Holarrhena antidysenterica*<sup>26</sup> and *Ruta graveolens*<sup>17</sup> revealed that the anti-diarrheal activity is due to the presence of tannins. Flavonoids are conveyed to inhibit the release of autacoids and prostaglandins, thus prevent the motility and secretion induced by castor oil<sup>6</sup>. Studies on the plants such as *C. blanchetianus* Baill, *C. rhamnifolius* Willd, and *C. argyroglossum* Baill have proved that the flavonoids and/tannins content were responsible for anti-diarrheal activity<sup>27</sup>. A recent study stated that the antidiarrheal activity of the extracts of *Osyris quadriartita* might be due to the highest phenolics and flavonoids contents detected in leaves that supports the antidiarrheal activity of phenolic compounds and tannins and flavonoids present in the *C. sativa* seeds<sup>28</sup>.

The current study revealed that both the extracts of *C. sativa* effectively inhibited the castor oil induced diarrhea due to its anti-motility and anti-secretory mechanism. This is proved from the observation of diarrheic feces and the reduction in frequency of stools in the tested groups at both doses of extracts. These effects were noticeable when comparing with the standard drug, Loperamide at 2mg/kg. The anti-diarrheal effect of *Castanea sativa* seeds might be due to the presence of phytoconstituents such as phenolic compounds and tannins and flavonoids.

**Table No.1: Colour, consistency and percentage yield of *Castanea Sativa* seeds extracts**

S.No	Type of Extract	Colour	Consistency	Percentage Yield (%)
1	Water Extract	Dark brown	Semi solid	17.8
2	Ethanolic Extract	Greenish yellow	Mucilagenous	3.15

**Table No.2: Preliminary phytochemical screening for *C. sativa* seeds extracts**

S.No	Phytoconstituents	Water Extract	Ethanol Extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Proteins	+	+
4	Phenolic compounds	+	+
5	Tannins	+	+
6	Flavonoids	+	+
7	Terpenoids	+	+
8	Sterols	+	+
9	Lipids and fatty acids	+	+

+ = Present, - = Absent

**Table No.3: Anti-diarrheal activity of *C. sativa* seeds extracts on castor oil induced diarrheal model**

Group	Treatment	Total number of feces	Weight of feces (gm)					Inhibition of diarrhea (%)
			1 h	2 h	3 h	4 h	5 h	
I	Castor oil (1ml)	15	1.87 ± 0.12	1.9 ± 0.2	2.06 ± 0.21	1.93 ± 0.20	1.83 ± 0.08	-
II	Standard drug + Castor oil	7	2.19 ± 0.01	1.18 ± 0.06*	0.75 ± 0.15**	0.7 ± 0.1**	0.7 ± 0.01***	61.74
III	Water extract (200 mg/kg) + Castor oil	10	1.44 ± 0.05*	1.13 ± 0.06*	1.10 ± 0.05*	0.8 ± 0.1**	0.59 ± 0.03***	67.76
IV	Water extract (400 mg/kg) + Castor oil	8	1.18 ± 0.06**	0.41 ± 0.08***	0.35 ± 0.04***	0.35 ± 0.03***	0.33 ± 0.02***	81.97
V	Ethanol extract (200 mg/kg) + Castor oil	14	1.65 ± 0.11	1.13 ± 0.03**	1.03 ± 0.01**	0.6 ± 0.08***	0.54 ± 0.08***	70.49
VI	Ethanol extract (400 mg/kg) + Castor oil	12	1.81 ± 0.07	0.74 ± 0.05***	0.71 ± 0.07***	0.68 ± 0.05***	0.52 ± 0.03***	71.58

\*\*\*P&lt;0.001, \*\*P&lt;0.01 and \*P&lt;0.05 statistically (Mean + SEM) significant from control group (n=4)

**CONCLUSION**

The findings of this study revealed that the water and ethanolic extracts of *C. sativa* possessed significant anti-diarrheal activity on castor oil induced diarrhea model. Furthermore, the water extract showed high spectrum of anti-diarrheal activity than ethanolic extract. The results scientifically support the anti-diarrheal activity of *Castanea sativa* seeds as a natural anti-diarrheal agent in treating diarrhea. The anti-diarrheal effect of *Castanea sativa* seeds might be due to the presence of phytoconstituents like flavonoids, phenolic compounds and tannins. However, more detailed phytochemical investigation is important to describe the bioactive compounds which might be responsible for anti-diarrheal activity of *Castanea sativa* seeds that will lead to further explore the possible mode of action at molecular level.

**ACKNOWLEDGEMENT**

The authors are thankful to the management, KPJ Healthcare University College, Kota Seriemas, Nilai, Negeri Sembilan, Malaysia, for providing facilities, technical assistance, funding and support.

**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

**BIBLIOGRAPHY**

1. Haque A, Zaman A, Hossain M, Sarker I and Islam S. Evaluation of antidiarrhoeal and insecticidal activities of ethanol extract and its fractions of *Dendrophthoe falcata* leaves, *International Journal of Pharmaceutical Sciences and Research*, 5(9), 2014, 3653-3663.
2. Meite S, N'Guessan J D, Bahi C, Yapi H F.,

- Djaman A J and Guede G F. Antidiarrheal activity of the ethyl acetate extract of *Morinda morindoides* in rats, *Tropical Journal of Pharmaceutical Research*, 8(3), 2009, 201-207.
3. Kaplan W, Wirtz V J, Mantel T A, Stolk P, Duthey B and Laing R. Priority medicines for Europe and the world 2013 update. *World Health Organization (WHO)*, 2013, 1-246.
  4. Fischer W C L, Perin J, Aryee M J, Boschi P, C and Black R E. Diarrhea incidence in low and middle income countries in 1990 and 2010: a systematic review, *BMC Public Health*, 12(1), 2012, 220.
  5. Bakare R I, Magbagbeola O A, Akinwande A I, Okunowo O W and Green M. Antidiarrhoeal activity of aqueous leaf extract of *Momordica charantia* in rats, *Journal of Pharmacognosy and Phytotherapy*, 3(1), 2011, 1-7.
  6. Hossain H, Kanti D S, Hira A, Howlader S I, Ahmed A and Sultana S. Evaluation of antidiarrhoeal potential of the ethanolic extract of three Bangladeshi medicinal plants, *International Journal of Pharmaceutical and Phytopharmacological Research*, 1(6), 2012, 371-374.
  7. Sarin R and Bafna P. Herbal anti-diarrheal: a review, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 3(2), 2012, 1-13.
  8. Ashok P and Upadhyaya K. Tannins are astringent, *Journal of Pharmacognosy and Phytochemistry*, 1(3), 2012, 45-50.
  9. Koeleman E. Sweet chestnut tannins in animal diets, *All About Feed*, 1(5), 2010, 22-23.
  10. Conedera M, Tinner W, Krebs P, Rigo D and Caudullo G. *Castanea sativa* in Europe : Distribution, habitat , usage and threats, *European Atlas of Forest Tree Species*, 2016, 78-79.
  11. Chiarini A, Micucci M, Malaguti M, Budriesi R, Ioan P, Lenzi M and Hrelia S. Sweet chestnut (*Castanea sativa* Mill) bark extract : Cardiovascular activity and myocyte protection against oxidative damage, *Oxidative Medicine and Cellular Longevity*, Article ID 471790, 2013, 1–10.
  12. Zivkovic J, Zekovic Z, Mujic I, Tumbas V, Cvetkovic D and Spasojevic I. Antioxidant properties of phenolics in *Castanea sativa* Mill. Extracts, *Food Technology and Biotechnology*, 47(4), 2009, 421-427.
  13. Anandarajagopal K, Sudhahar D, Ajaykumar T and Muthukumaran G. Evaluation of CNS depressant activity of aerial parts of *Basella alba* Linn, *IJPI's Journal of Pharmacology and Toxicology*, 1(5), 2011, 1-6.
  14. Ayoola G, Coker H, Adesegun S, Adepoju-Bello A, Obaweya K, Ezennia E and Atangbayila T. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria, *Tropical Journal of Pharmaceutical Research*, 7(3), 2008, 1019-1024.
  15. Rajamanickam V, Rajasekaran A, Anandarajagopal K, Sridharan D, Selvakumar K and Rathinaraj B S. Anti-diarrheal activity of *Dodonaea viscosa* root extracts, *International Journal of Pharma and Bio Sciences*, 1(4), 2010, 182-185.
  16. Sunilson A J, Anandarajagopal, Kumari A V A G and Mohan S. Anti-diarrhoeal activity of leaves of *Melastoma malabathricum* Linn, *Indian Journal of Pharmaceutical Sciences*, 71(6), 2009, 690-694.
  17. Akter S, Sarker, A and Hossain, M S. Antidiarrhoeal activity of rind of *Punica granatum*, *International Current Pharmaceutical Journal*, 2(5), 2013, 101-104.
  18. Rahman M K, Barua S, Islam M F, Islam M R, Sayeed M A, Shahnaj M P and Islam M E. Studies on the anti-diarrheal properties of leaf extract of *Desmodium puchellum* studies, *Asian Pacific Journal of Tropical Biomedicine*, 3(8), 2013, 639-643.
  19. Komal, Kumar S and Rana A C. Herbal

- approaches for diarrhoea: a review, *International Research Journal of Pharmacy*, 4(1), 2013, 31-38.
20. Sisay M, Engidawork E and Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extracts of *Myrtus communis* Linn (Myrtaceae) in mice model, *BMC Complementary and Alternative Medicine*, 17(1), 2017, 103.
21. Pandey P, Mehta A and Hajra S. Antidiarrhoeal activity of ethanolic extracts of *Ruta graveolens* leaves and stem, *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), 2012, 65-68.
22. Bhamadevi R. Screening of bioactive compounds from the leaves of *Moringa concanensis* Nimmo, *International Journal of Innovative Research in Science, Engineering*, 4(10), 2015, 9702-9709.
23. Sanni F S, Hamza H G and Onyeyili P A. Antidiarrheal activity of fractions from aqueous extract of *Detarium senegalense*, 61(2), 2015, 30-40.
24. Ezeja I M, Ezeigbo I I, Madubuike K G, Udeh N E, Ukwani I A, Akomas S C and Ifenkwe D C. Antidiarrheal activity of *Pterocarpus erinaceus* methanol leaf extract in experimentally-induced diarrhea, *Asian Pacific Journal of Tropical Medicine*, 5(2), 2012, 147-150.
25. Konate K, Yomalan K, Sytar O and Brestic M. Antidiarrheal and antimicrobial profiles extracts of the leaves from *Trichilia emetica* Vahl. (Meliaceae), *Asian Pacific Journal of Tropical Biomedicine*, 5(3), 2015, 242-248.
26. Sharma D K, Gupta V K, Kumar S, Joshi V, Shankar R, Mandal K and Singh M. Evaluation of antidiarrheal activity of ethanolic extract of *Holarrhena antidysenterica* seeds in rats, *Veterinary World*, 8(12), 2015, 1392-1395.
27. Siqueira C F D Q, Cabral D L V, Da Silva P S T J, De Amorim, E L C, De Melo, J G, De Sousa A T A and De Albuquerque U P. Levels of tannins and flavonoids in medicinal plants: evaluating bioprospecting strategies, *Evidence-Based Complementary and Alternative Medicine*, Article ID 434782, 2012, 1-7.
28. Meseret Y T, Mohammedbirhan A and Jibril S Y. Evaluation of *in vivo* antidiarrheal activity of 80% methanolic leaf extract of *Osyris quadripartita* Decne (Santalaceae) in swiss albino mice, *Journal of Evidence Based Integrative Medicine*, 24, 2019, 1-9.

**Please cite this article in press as:** Anandarajagopal Kalusalinagm et al. Evaluation of anti-diarrheal activity of the extracts of *Castanea sativa* seeds, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 7(2), 2019, 701-707.