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EVALUATION OF ANTI-DIARRHEAL & ANTI-SPASMOLYTIC AND INVITRO ANTI OXIDANT PROPERTIES OF METHANOLIC EXTRACTS OF LANTANA CAMARA AND MYRTUS COMMUNIS

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ABSTRACT

Presently, around 25% of medications produced in the globe, are extracted straight from plants or herbs contains at least one active component. It is acknowledged that the popular of herbal medicines employed as antidiarrheals have antispasmodic characteristics consequential in delaying gastrointestinal procedures, inhibiting gut motility, initiating absorption of water and dropping electrolyte discharge in the process, and these biological activities might explain the benefits of by means of specific herbal medicines in the management and treatment of diarrhoea. The main aim of the study is to screen Myrtus communis and Lantana camara extracts for their anti-diarrheal and anti-spasmodic activity. This study was intended to assess the antidiarrheal activity of the selected herbs by means of Swiss albino mice models against castor oil stimulated diarrhea, castor oil stimulated gastrointestinal transit, and castor oil stimulated accumulation of gastrointestinal fluid. In the execution of antimotility, diarrhea, and anti-secretory agent are seem to be the chief stay agents utilized to drop off the pathophysiologic circumstances accountable for the progress of diarrhea. The inhibitory force of loperamide on acetylcholine cause inhibition of discharge intervened with acetylcholine. As an outcome, loperamide lessen every day fecal volume, reduce fluid and loss of electrolyte, and may perhaps augment viscosity of stool and bulk density. Coming to the Myrtus communis and Lantana camara, the result of the study verified that the plant extracts was caused a noteworthy impediment in the onset of diarrhea, reduction in the occurrence of output of wet fecal and total fecal, along with diminish in the mean weight of wet feces and output of total fecal that were caused by means of castor oil.. The plants extracts established a noteworthy delay in the onset of diarrhea, abridged the occurrence of wet feces and also endowed with noteworthy anti-secretory effects at all doses assessed experimentally. And also, the plants extracts signified the antimotility activity at its higher doses.

Keywords: Lantana camara, myrtus communis, anti-diarrhea, anti-spasmodic

INTRODUCTION:

Herbal plants importance:

The utilisation of plants in the treatment of certain human diseases is evidence of man's ingenuity. The contribution of these plants to the therapeutic arsenal in the fight against diseasedates back several centuries, and has, to a certain extent, been documented by the ancient Chinese, Indian and North African civilisations[1-3]. As the fact that traditional health care is highly soughtafter in terms of certain cultural elements in the lives of the individuals in the societies[4]. In southern Africa, a large proportion of the population still uses traditional remedies. More than 700 plant species are being traded for medicinal purposes throughout South Africa, in theinformal medicinal plant market. It is evident that, even though scientific advances have been made in our quest to understand the physiology of the body, biotechnology and the treatment of disease, natural products remain a crucial component of the comprehensive health care strategy for the future[5,6].

The World Health Organization (WHO) defines traditional medicine as the "diverse health practices, approaches, knowledge and beliefs incorporating plant-, animal- and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness". Traditional medicine utilises biological resources and the indigenous knowledge of traditional plant groups, the later being conveyed verbally from generation to generation[7]. The Greek physician Dioscorides (AD 70) compiled an extensive listing of medicinal herbs and their virtues. This was originally written in Greek, and later translated into Latin as De Materia Medica, and remained the authority in medicinal plants for over 1500 years[2].

Presently, around 25% of medications produced in the globe, are extracted straight from plants or herbs contains at least one active component. Based on who around 80% of the population of world's depend on drugs derivative from medicinal plants and they utilized it for therapy[7].

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Herbal plants are utilized for managing and treating gastrointestinal ailments (cholera, diarrhoea, and dysentery), followed by means of sexually transmitted infections, cough, cold, sore throat and gynaecological complications[8].

AIM AND OBJECTIVES:

Aim:

The main aim of the study is to screen the selected herbal extracts for their anti-diarrheal, anti-spasmodic activity and in-vitro antioxidant properties of methanolic exracts of Lanata camara and Myrtus communis .

Objectives:

To prepare the methanolic extracts of leaves of selected medicinal plants. In-vitro antioxidant property Phytochemical screening of methanolic extracts To evaluate the parameters like Determination of antidiarrheal activity Gastrointestinal motility In vivo antidiarrheal index

MATERIALS AND METHODS:

A. Plant Collection and Extraction

i)Fresh plants material parts were collected and authenticated through registered botanist Dr. Madhavachetty, SVU, Tirupati. Then the plants material was extracted using methanol solvent by means of utilizing soxhlet extractor awaiting the color in siphon tube become colorless. Collected liquid extract was filtered out and extract concentrated by means of utilizing rota evaporator. Resulting extract was packed in a container and amassed in refrigerator.

ii) Later than extraction phytochemical screening and bio active constituents characterization study was finished by means of employing suitable methods[95].

Qualitative analysis methods Table 1:
Qualitative analysis methods

S.No.	Phytochemical Constituent	Tests
1	Alkaloids	Mayer's test
		Dragendroff's test
		Hager's test
		Wagner's test
2	Carbohydrates	Molisch's test
3	Reducing Sugars	Fehling's Test
		Benedicts test
4	Saponins	Foam test
		Forth test
5	Phytosteroids	Salkowski's test
		Liebermann Burchard's test
6	Phenols	Ferric chloride test
		Lead acetate test
7	Tannins	Ferric chloride test

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8	Flavonoids	Lead acetate test
		Alkaline reagent test
9	Cardiac Glycosides	Killer- Kallani test
10	Proteins &Aminoacids	Millons test
		Biuret test
		Ninhydrin test
11	Terpenoids	Salkowski's test
12	Fixed oils & fats	Spot test
		Saponification test
13	Gum & Mucilage	Ruthenium red solution

A. In-vitro antioxidant study

Catalase is an enzyme that decomposes hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2). Chemically, it is a hemoprotein structurally similar to hemoglobin.

Catalase

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Hydrogen Peroxide → Water + Oxygen

MATERIALS

- Catalase assay buffer - 25 ml
- Catalase positive control - 2 µl
- Hydrogen peroxide(0.88M) - 25 µl
- Stop solution - 1 ml
- Oxired probe - 200 µl
- HRP (lyophilized) - 1 vial

PROCEDURE

Prepare Catalase Reaction for each sample, positive control and sample HC wells by mixing1.5 µL fresh 1 mM H2O2 solution with 10.5 µL Catalase Assay Buffer. Prepare a master mix to ensure consistency

Add 12 µL diluted H2O2 solution into each sample, positive control and sample HC wells.

Incubate reaction at 25ºC for 30 minutes.

Add 10 µL Stop Solution to each sample and positive control wells. Do Stop Solution tostandard dilution or to Sample HC well Prepare 50 µL of Developer Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix ofthe Developer mix to ensure consistency.

Add 50 µL of Developer Mix into each standard, sample, sample HC and positive controlwells.

Mix and incubate at 25ºC for 10 min protected from light.

Measure output immediately at Ex/Em = 535/587 nm on a microplate reader.

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Catalase activity in the test samples is calculated by using formula.

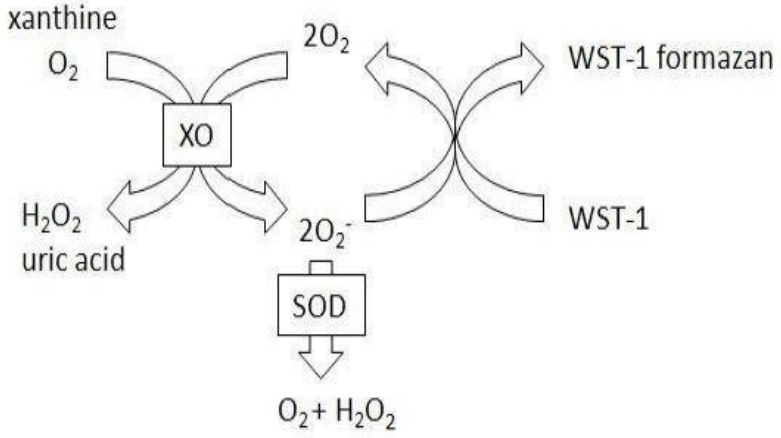
SUPEROXIDE DISMUTASE (SOD) ASSAY

Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide ($O2^{-}$) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging andis degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen.

$2O_2 + 2H_3 \qquad O_2 + H_2O_2 + 2H_2O$

PRINCIPLE

SOD enzymes deal with the superoxide radical by alternately adding or removing an electron from the superoxide molecules it encounters, thus changing the $O2^{-}$ into one or two less damaging species, either molecular oxygen (O2) or hydrogen peroxide (H2O2).



LIPID PEROXIDATION ASSAY

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (- CH2-) that possess especially reactive hydrogen atoms. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination. The chemicalproducts of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs).

PRINCIPLE

During peroxide formation form fatty acid containing methylene-interrupted double bonds, that is, those found in the naturally occurring polyunsaturated fatty acids, Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate future peroxidationand thus has potentially devastating effects. The whole process can be depicted as follows.

PROPAGATION

The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen,thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or acyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radicalreacted.

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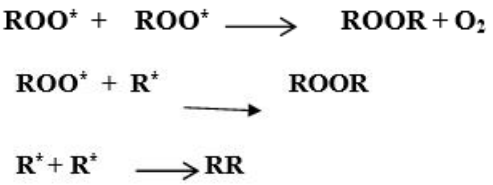
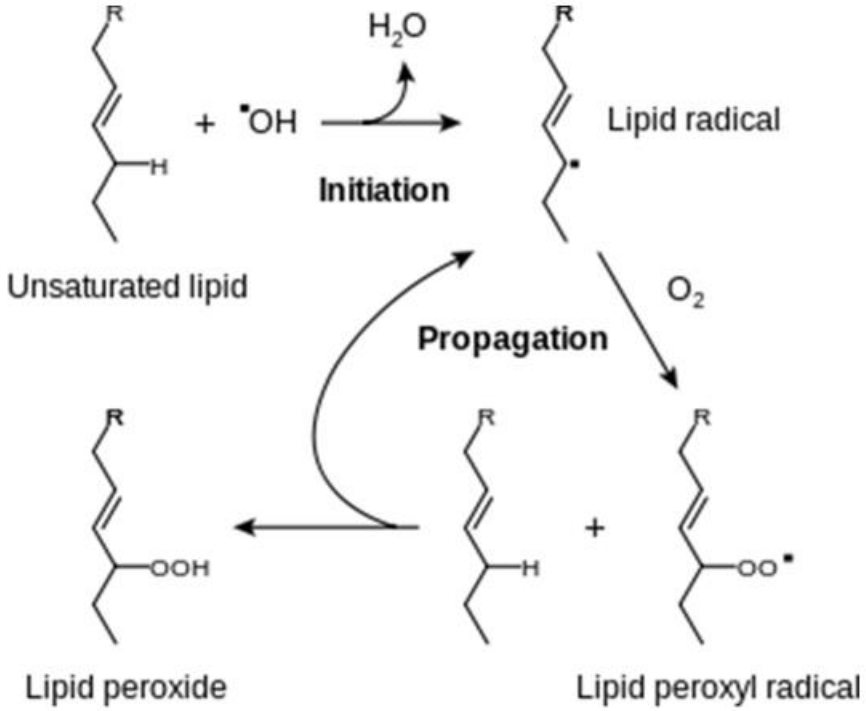
TERMINATION

It is marked by the slowing or stopping of reactions, completion of making unreactive compounds or when an antioxidant is added or encountered. There are two basic types of oxidative by products, primary and secondary.





MATERIALS

MDA(malondialdehyde) Lysis Buffer - 25ml

Phosphotungstic Acid Solution - 12.5 ml

BHT - 1ml

TBA (Thiobarbituric acid) Solution - 4 vials

MDA Standard - 100 µl

Experimental animals:

Six weeks old Male wistar rats were procured from Nirmala college of Pharmacy. All experimentation and procedures carried out on the animals (rats) were approved by the Institutional Ethics Committee of Nirmala College of Pharmacy. Rats were housed in a proscribed temperature of room at 25

± 1◦C under standard mentioned surroundings (12-h dark-light cycle). They were housed in a

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polypropylene cage and presented food and water ad libitum. Animals were quarantined and become familiarized laboratory conditions for 7 days before scheduled to study initiation. Animals were observed for common health and suitability for investigation during this phase.

Acute Toxicity

Before beginning of animal investigation, the acute toxicity study (according to OECD guideline) was carried out to established effective dose of test compounds. Based on findings of acute toxicity, the lead extracts were tested in suitable diarrhea model[96].

In-Vivo Study[97-100]:

Experimental Animals:

Swiss albino mice weighing 20 to 30 g of age 6 to 8weeks were employed for theexperimentation. The mice were housed in plastic cages at 22+3Centigrade and on a 12-hourlight and 12 hr dark cycle amid accessible to food pellet and water ad libitum. Good hygienicconditions were maintained by means of constant cleaning and exclusion of feces as of cages3times a week. The mice were habituated to laboratory surroundings for 1 week proceeding to the experimentation. Food was taken out 18 hrs proceeding to the commencement of all the experiments. The care and treatment were based on international guidelines for the utilizationand upholding of experimental animals.

Grouping and Dosing of Animals:

Mice were randomly assigned into 6 groups of 2 plant test extracts (2 dose groups for each extract) treated, 1 disease control group and standard group with 5 mice per each group.

Table 2: Grouping of Animals

Group name	**Treatment**	**Dose / Route**
Control	Normal Diet	
Diseasecontrol	*Distilled water + Castor oil*	3 mg/kg body weight dissolved insaline. Single dose treatment using oral gavage.
positive control	standard drug + *Castor oil* + loperamide	10 ml/kg / Through oral gavage
Test Group- 1	*Castor oil* + 200 mg/kg of Methanolic extract of *Lantana camara*	200mg/kg Body Wt./ ThroughOral route Dissolved in 0.9% Saline

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Test Group- 2	*Castor oil* + 400 mg/kg of Methanolic extract of Lantana camara	400mg/kg Body Wt. / Through Oral route Dissolved in 0.9%Saline
Test Group- 3	*Castor oil* + 200 mg/kg of Methanolic extract of *Myrtuscommunis*	200mg/kg Body Wt. /Through Oral route Dissolved in 0.9%Saline
Test Group- 4	*Castor oil +400 mg/kg of Methanolic extract of Myrtuscommunis*	400mg/kg Body Wt. / Through Oral route Dissolved in 0.9% Saline

The in vivo antidiarrheal index (ADI) for the plants extracts and standard drug was determined by means of employing the following formula:

$$ADI=\sqrt[3]{(D\ freq \times G\ meq \times P\ freq)}$$

Dfreq= (Mean onset of diarrhea in treated group-Mean onset of diarrhea in negativecontrol/ Mean onset of diarrhea in the negative control group) x100

Statistical Analysis:

All the results were articulated as Mean ± S.E.M. The inter group disparity amongst the various groups was analyzed by means of one way analysis of variance (ANOVA) using the Graph Pad Prism, version 5.0. Results were taken as statistically significant when $p < 0.05$

RESULTS

1. **Phytochemical ingredient present in Methanol extract of *Myrtuscommunis* and *Lantanacamara***

Table 2: Particulars of qualitative phytochemical assessment of methanol extract ofselected plants.

S.No.	**Phytochemical Constituent**	**Tests**	***Myrtuscommunis***	***Lantana camara***
1	**Alkaloids**			
		Mayer's test	+	+
		Wagner's test	+	+
2	**Carbohydrates**			

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10	**Proteins & Amino acids**			
		Biuret test	-	-
		Ninhydrin test	-	-
		Molisch’s test	+	+
3	**Reducing Sugars**			
		Fehling’s Test	+	-
6	**Phenols**			
		Ferric chloride test	+	-
		Lead acetate test	-	-
7	**Tannins**			
		Ferric chloride test	+	-
8	**Flavonoids**			
		Lead acetate test	-	-
		Alkaline reagent test	-	-
9	**Glycosides**			
		Borntrager's test	+	+
		Legals test	+	+
		Benedicts test	+	-
4	**Saponins**			
		Foam test	+	+
		Haemolysis test	+	+
5	**Flavones and Flavonoids**			
		Caddy's test	+	+
		Shinoda test	+	+

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11	**Triterpenoids**			
		Tin+thionyl chloride	-	-
12	**Fixed oils & fats**			
		Spot test	-	-
		Saponification test	-	-

The strapping presence of needed phytochemicals in Methanol extract was witnessed. And additional investigations, extracts of *Myrtuscommunis*and *Lantana camara* were carried out.

Toxicity study:

In the current exploration, the Methanol extracts of *Myrtuscommunis* and *Lantana camara* were levied for studies of acute toxicity.For the determination of LD_{50} dose, Methanol extract of *Myrtuscommunis* and *Lantana camara* was given up to dose o f 2 gm/kg b.w. and extracts did not exhibited any sort of mortality, that's why 1/5th (400mg),1/10th (200mg)of most dose given

INVITRO ANTI OXIDANT STUDIES: CATALASE ASSAY

Table no: 03

S.NO	DRUG FORMULATIONS	ABSORBANCE VALUE	% SCVENGING ACTIVITY
1	CONTROL	0.345	-
2	ASCORBIC ACID	0.456	90.5%
3	*Myrtus communis* 100µg/ml	0.386	54.3%
4	*Lantana camara* 100µg/ml	0.401	57.2%

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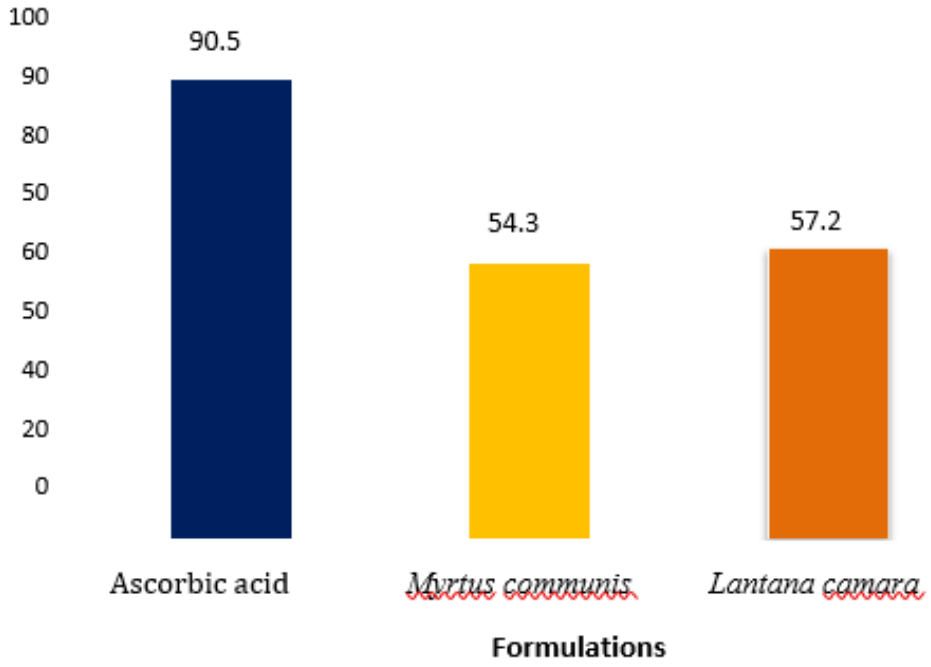
RESULTS OF CATALASE SCAVENGING ACTIVITY



Acute Toxicity Assessment

Table 6: Acute Toxicity Assessment

S.no.	Code	Toxicity		Time Of Death	Observation									
		Onset	Stop		Skin colour	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Leth
1.	MCG	X	X	x	x	x	x	X	x	x	x	x	X	x
2.	MCG	X	X	x	x	x	x	X	x	x	x	x	X	x

(TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LETH-Lethargy)

× = Negative Ø = Positive

***Invivo* Antidiarrheal Index**

Group name	Onset of diarrhea (min)	Number of wet feces	Total number of Feces	Average weight of the feces (g)	Average wet of total feces (g)	% of inhibitionof defecation	%WWFO	%WTFO
Disease control	82.98 ±1.64	5.4 ± 0.65	6.5 ± 0.47	1.21 ± 0.08	1.37 ± 0.06	-	-	-

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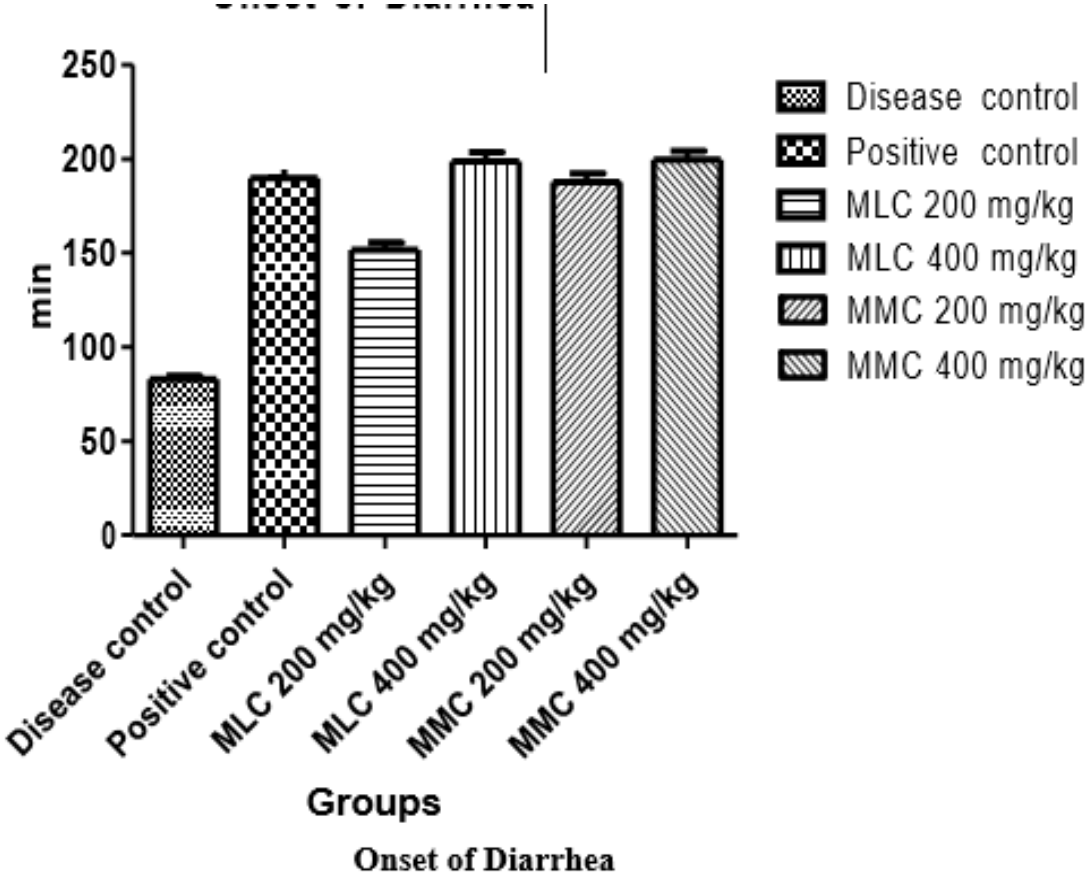
positive control	189.9 ±4.6	0.97 ± 0.02	1.57 ± 0.12	0.11 ± 0.01	0.15 ± 0.02	81.12	7.14	9.27

	151.67 ± 3.8	1.76 ± 0.05	3.17 ± 0.08	0.16 ± 0.02	0.2 ± 0.01	66.92	13.87	15.56
Test Group- 2	198.67 ±4.9	1.15± 0.03	1.75 ± 0.06	0.13 ± 0.01	0.18 ± 0.01	77.94	8.76	14.15
Test Group- 3	187.79 ±4.7	1.35± 0.03	2.18±0.06	0.13 ± 0.02	0.19 ± 0.01	74.5	10.38	14.96
	199.72 ±4.5	1.13± 0.02	1.7 ± 0.05	0.12 ± 0.01	0.16 ± 0.01	78.67	8.89	13.93



Onset of Diarrhea

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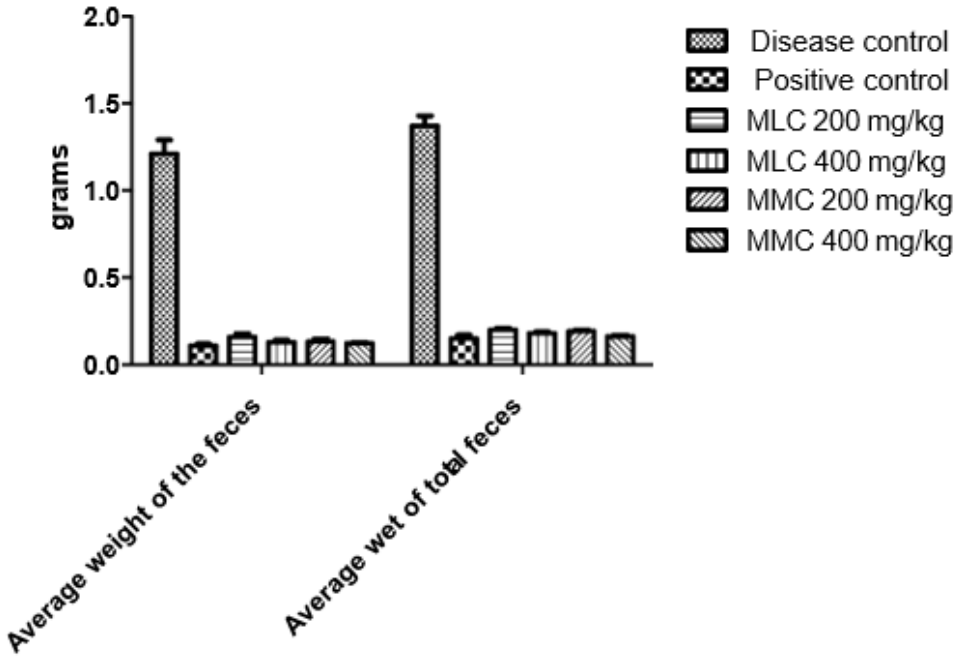

Avg. wt of Faces Vs Total Avg. wt.

CONCLUSION:

The study investigated the acute toxicity of the plants extracts, the plants *Myrtuscommunis* and *Lantana camara* are instituted to be nontoxic and its LD50 is higher than 2000 mg/kg, which ensures the safe use of the plants extracts. The *Myrtuscommunis* and *Lantana camara* exhibited antidiarrheal effect on evaluation in animal models by means of Swiss albino mice. The plants extracts established a noteworthy delay in the onset of diarrhea, abridged the occurrence of wet feces and also endowed with noteworthy anti-secretory effects at all doses assessed experimentally. And also, the plants extracts signified the antimotility activity at its higher doses. Though additional investigations are warranted by means of various anti- diarrheal models and solvents, at this stage the findings of the investigation established the antidiarrheal activity of the plants.

Conflict of Interest: The author

REFERENCES

1. https://data.unicef.org/topic/child-health/diarrhoeal-disease/

2. Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, Hennessy T, Griffin PM, DuPont H, Sack RB, Tarr P, Neill M, Nachamkin I, Reller LB, Osterholm MT, Bennish ML and Pickering LK: Infectious Diseases Society of, A. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001; 32: 331-351.

3. Riddle MS, DuPont HL and Connor BA: ACG clinical guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. The American journal of gastroenterology 2016; 111(5): 602-22.

4. Rimoldi SG, Stefani F, Pagani C, Chenal LL, Zanchetta N, Di Bartolo I, et al.

Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents. Arch Virol. 2011; 156(9): 1583–9.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

5. Pfeiffer ML, DuPont HL, Ochoa TJ. The patient presenting with acute dysentery--a systematic review. J Infect. 2012;64(4):374–86.
6. Ede R. Causes and recommended management of acute diarrhoea. Prescriber. 2014; 25(8): 17-23.
7. Maroyi A. Treatment of diarrhoea using traditional medicines: Contemporary research in South Africa and Zimbabwe. African Journal of Traditional, Complementary and Alternative Medicines. 2016; 13(6): 5-10.
8. Chen J, Wan CM, Gong ST, Fang F, Sun M, Qian Y, Huang Y, Wang BX, Xu CD, YeLY, Dong M, Jin Y, Huang ZH, Wu QB, Zhu CM, Fang YH, Zhu QR, Dong YS. Chinese clinical practice guidelines for acute infectious diarrhea in children. World JPediatr. 2018; 14(5): 429-436.

1. Shoba FG and Thomas M: Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhea. Journal of Ethnopharmacology 2001; 76: 73-76.
2. Imam MZ, Sultana S and Akter S: Antinociceptive, antidiarrhoeal and neuropharmacological activities of Barringtonia acutangula. Pharmaceutical Biology 2012; 50(9): 1078-84.

3. Null C, Stewart CP, Pickering AJ, Dentz HN, Arnold BF, Arnold CD, Benjamin-Chung J, Clasen T, Dewey KG, Fernald LCH, Hubbard AE, Kariger P, Lin A, Luby SP, Mertens A, Njenga SM, Nyambane G, Ram PK, Colford JM. Effects of water quality,sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial. Lancet Glob Health. 2018; 6(3): e316-e329.
4. Wenzl HH. Diarrhea in chronic inflammatory bowel diseases. Gastroenterol. Clin.

North Am. 2012; 41(3): 651-75.

5. Lopman BA, Steele D, Kirkwood CD, Parashar UD. The Vast and Varied Global Burden of Norovirus: Prospects for Prevention and Control. PLoS Med. 2016; 13(4):e1001999.
6. Burisch J, Munkholm P. The epidemiology of inflammatory bowel disease. Scand. J.Gastroenterol. 2015; 50(8): 942-51.
7. Szilagyi A, Ishayek N. Lactose Intolerance, Dairy Avoidance, and Treatment Options.

Nutrients. 2018; 10(12): 312-319.

8. Nikfarjam M, Wilson JS, Smith RC. Australasian Pancreatic Club Pancreatic Enzyme Replacement Therapy Guidelines Working Group. Diagnosis and management of pancreatic exocrine insufficiency. Med. J. Aust. 2017; 207(4): 161-165.
9. Schiller LR. Management of diarrhea in clinical practice: strategies for primary care physicians. Rev Gastroenterol Disord. 2007; 7 Suppl 3: S27-38.

1. Gauchan E, Malla KK. Relationship of Renal Function Tests and Electrolyte Levels with Severity of Dehydration in Acute Diarrhea. J Nepal Health Res Counc. 2015; 13(29): 84-9.
2. Santos JI. Nutritional implications and physiologic response to pediatric diarrhea.

Pediatr Infect Dis. 1986; 5(1 Suppl): S152-4.

3. Dekate P, Jayashree M, Singhi SC. Management of acute diarrhea in emergency room.

Indian J Pediatr. 2013; 80(3): 235-46.

4. Schiller LR. Antidiarrheal Drug Therapy. Curr Gastroenterol Rep. 2017; 19(5): 18-24.

5. Lau CS, Chamberlain RS. Probiotics are effective at preventing Clostridium difficile- associated diarrhea: a systematic review and meta-analysis. Int J Gen Med. 2016; 9: 27- 37.
6. Bolia R. Approach to "Upset Stomach". Indian J Pediatr. 2017; 84(12): 915-921.

7. Kakoullis L, Papachristodoulou E, Chra P, Panos G. Shiga toxin-induced haemolytic uraemic syndrome and the role of antibiotics: a global overview. J. Infect. 2019; 79(2): 75-94.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

8. Prüss-Ustün A, Wolf J, Bartram J, Clasen T, Cumming O, Freeman MC, Gordon B, Hunter PR, Medlicott K, Johnston R. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. Int J Hyg Environ Health. 2019; 222(5): 765-777.

a. Alem G, Mekonnen Y, Tiruneh M and Mulu A, In vitro antibacterial activity of crude preparation of myrtle (Myrtus communis) on common human pathogens,Ethiop Med J, 2008; 46(1): 63-69.

b. Mohammadi R, Esfahani S H M, Shadzi S and Moattar F, Antifungal activity of Myrtus communis L. esssential oil against clinical isolates of Aspergillus, J IsfahanMedical School, 2008; 26(89): 105-111.

c. Deuruaz D and Raynaud J, Evaluation of the molluscicidal properties of Myrtus communis Linn., Phytother Res, 1993; 7(6): 428-430.

d. Traboulsi A F, Taoubi K, El-Haj S, Bessiere J M andRammal S, Insecticidal properties of essential plant oils against the mosquito Culex pipiens molestus (Diptera: Culicidae), Pest Manag Sci, 2002; 58(5): 491-495.

e. Feisst C, Franke L, Appendino G and Werz O, Identification of molecular targets of the oligomeric nonprenylated acylphloroglucinols from Myrtus communis and their implication as anti-inflammatory compounds, J Pharmacol Exp Ther, 2005; 315(1): 389-396.

f. Serce S, Ercisli S, Sengul M, Gunduz K and Orhan E, Antioxidant activities and fatty acid composition of wild grown myrtle (Myrtus communis L.) fruits, Phcog Mag, 2010; 6: 9-12.

g. Elfellah M S, Akhter M H and Khan M T, Antihyperglycaemic effectof an extract of Myrtus communis in streptozotocin-induced diabetes in mice, J Ethnopharmacol, 1984; 11: 275-281.

h. Mimica-Dukic N, Bugarin D, Grbovic S, Mitic-Culafic D, Vukovic-Gacic B, Orcic D, Jovin E and Couladis M, Essential oil of Myrtus communis L. As a potential antioxidant and antimutagenic agents, Molecules, 2010; 15(4):2759-2770.

i. Tretiakova I, Blaesius D, Maxia L, Wesselborg S, Schulze-Osthoff K, Cinatl JJr, Michaelis M and Werz O, Myrtucommulone from Myrtus communis induces apoptosis in cancer cells via the mitochondrial pathway involving caspase-9, Apoptosis, 2008; 13(1): 119-131.

9. Al-zohyri A M, Al-Jeboory A A and Jawad A L M, Cardiovascular and antimicrobial effect of Myrtus communis,Indian J Pharmacol, 1985; 17(4): 233-235.

10. Babaee N, Mansourian A, Momen-Heravi F, MoghadamniaA and Momen- Beitollahi J, The efficacy of a paste containing Myrtus communis (Myrtle) in the management of recurrent aphthous stomatitis: A randomized controlled trial, Clin Oral Invest, 2010; 14: 65-70.

11. Salouage I, Klouz A, Ferchichi H, Charfi R, Ouanes L, Boussaid M and Lakhal M, Effect of Myrtus communis L. on an experimental model of a rat liver ischemia- reperfusion, International Symposium on Medicinal and Aromatic Plants, ISHS ActaHortic, 2009: 853-862.

12. Sumbul S, Ahmad M A, Asif M, Saud I and Akhtar M, Evaluation of Myrtus communis Linn. berries (common myrtle) in experimental ulcer models in rats, Hum Exp Toxicol, 2010; 29(11): 935-944.

13. Twaij H and El-Jalil H A,Evaluation of Narcotic (Opioid like) analgesic activities of medicinal plants, Europ J Sci Res, 2009; 33(1): 179-182.

14. Bhakta D, Ganjewala D. Effect of leaf positions on total phenolics, flavonoids and proantho-cyanidins content and antioxidant activities in Lantana camara (L). Journal of Scientific Research. 2009; 1 (2): 363-369.

15. Mayee R, Thosar A, Evaluation of Lantana camara Linn. (Verbenaceae) for antiurolithiatic and antioxidant activities in rats. International Journal of Pharmaceutical and Clinical Research. 2011; 3 (1): 10-14.

1. Ganjewala D, Sam S and Khan KH. Biochemical compositions and antibacterial activities of Lantana camara plants with yellow, lavender, red and white flowers.EurAsian Journal of BioSciences. 2009; 3: 69-77.

2. Barreto FS. Antibacterial activity of Lantana camara Linn and Lantana montevidensis Brig extracts from Cariri-Ceará, Brazil. Journal of Young Pharmacists. 2010; 2 (1): 42-44.

3. Srivastava D, Singh P. Antifungal potential of two common weeds against plant pathogenic fungi-Alternaria sps. Asian Journal of Experimental Biological Sciences.2011; 2 (3): 525-528.

4. Tripathi S. Potential of Lantana camara Linn. Weed against wood destroying fungi.

Indian Forest. 2009; 135 (3): 403-411.

5. Dua VK, Pandey AC and Dash AP. Adulticidal activity of essential oil of Lantana camara leaves against mosquitoes. Indian Journal of Medical Research. 2010; 131: 434-439.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

6. Kumar MS, Maneemegalai S. Evaluation of Larvicidal Effect of Lantana Camara Linn. against mosquito species Aedesaegyptiand Culex quinquefasciatus. Advances in Biology Research. 2008; 2 (3-4): 39-43.
7. Hamotharan G. Antiulcerogenic effects of Lantana camara Linn. leaves On in vivo test models in rats. Asian Journal of Pharmaceutical and Clinical Research. 2010; 3 (3): 57-60.
8. Nayak BS. Evaluation of wound healing activity of Lantana camara L. - a preclinical study. Phytotherapy Research. 2009; 23 (2): 241-245.
9. Patel J, Kumar GS, Deviprasad SP, Deepika S, Qureshi MS. Phytochemical and anthelmintic evaluation of Lantana camara (l.) var. aculeate leaves against Pheretimaposthuma. Journal of Global Trends in Pharmaceutical Sciences. 2011; 2(1): 11-20.

1. Forestieri AM, Monforte MT, Ragusa S, Trovato A, Iauk L. Antiinflammatory, analgesic and antipyretic activity in rodents of plant extracts used in African medicine. Phytotherapy research. 1996; 10(2): 100-6.
2. Ganesh T, Sen S, Thilagam E, Thamotharan G, Loganathan T, Chakraborty R. Pharmacognostic and anti-hyperglycemic evaluation of Lantana camara (L.) var. aculeate leaves in alloxan-induced hyperglycemic rats. International Journal of Research in Pharmaceutical Sciences. 2010; 1(3): 247-52.
3. Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal activity of 80% methanolic leaf extract of Justicia schimperiana. Evidence-Based Complementary and Alternative Medicine. 2018; 2: 18-27.
4. Naher S, Aziz MA, Akter MI, Rahman SM, Sajon SR, Mazumder K. Anti-diarrheal activity and brine shrimp lethality bioassay of methanolic extract of Cordyline fruticosa (L.) A. Chev. leaves. Clinical Phytoscience. 2019; 5(1): 15-24.
5. Degu A, Engidawork E, Shibeshi W. Evaluation of the anti-diarrheal activity of the leaf extract of Croton macrostachyus Hocsht. ex Del.(Euphorbiaceae) in mice model. BMC Complementary and Alternative Medicine. 2016; 16(1): 37-39.
6. Teferi MY, Abdulwuhab M, Yesuf JS. Evaluation of in vivo antidiarrheal activity of80% methanolic leaf extract of Osyris quadripartita Decne (Santalaceae) in Swiss Albino Mice. Journal of evidence-based integrative medicine. 2019; 24: 25-35.
7. Wansi SL, Nguelefack-Mbuyo EP, Nchouwet ML, Miaffo D, Nyadjeu P, Wabo JP, Mbiantcha M, NKeng-Efouet PA, Nguelefack TB, Kamanyi A. Antidiarrheal activityof aqueous extract of the stem bark of Sapium Ellipticum (Euphorbiaceae). TropicalJournal of Pharmaceutical Research. 2014; 13(6): 929-35.
a. Afroz N, Hoq MA, Jahan S, Islam MM, Ahmed F, Shahid-Ud-Daula AF, Hasanuzzaman M. Methanol soluble fraction of fruits of Annona muricata possessessignificant antidiarrheal activities. Heliyon. 2020; 6(1): e03112.
b. Yadav AK, Tangpu V. Antidiarrheal activity of Lithocarpus dealbata. and Urena lobata.

Extracts: therapeutic implications. Pharmaceutical Biology. 2007; 45(3): 223-9.

c. Sini KR, Sinha BN, Rajasekaran A. Antidiarrheal activity of Capparis zeylanica leafextracts. Journal of advanced pharmaceutical technology & research. 2011; 2(1): 39-47.
d. Tagne MF, Rékabi Y, Noubissi PA, Olivier G, Fankem HA, Wambe H, Kamgang R.Evaluation of antidiarrheal activity of aqueous leaf extract of Anogeissus leiocarpus on castor oil-induced diarrhea in rats. American Journal of Biomedical Science &Research. 2019; 3(1): 27-34.
e. Dash PR, Nasrin M, Raihan SZ, Ali MS. Study of antidiarrhoeal activity of two medicinal plants of Bangladesh in castor-oil induced diarrhoea. International Journal of Pharmaceutical Sciences and Research. 2014; 5(9): 38-43.
f. Nadkarni K M, Indian Materia Medica, 3rd Edn, Popular Prakashan Pvt. Ltd., Bombay, vol. 1, 1989, p. 838.
g. Medicinal Plants of India, Indian Council of Medical research, New Delhi, Vol. II, 1987, pp. 310-311.
h. Kirtikar KR and Basu BD, Indian Medicinal Plants, 3rd Edn, International Book Distributors, Dehra Dun, Vol. II, 1988, 1040-1042.
i. Stuart M, The Encyclopedia of Herbs and Herbalism, 3rdEdn, 1994, pp. 52, 136.

j. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products Raw Materials Series, Publications and Information Directorate,Council of Scientific & Industrial Research, New Delhi, India, Vol. VI, 1962, pp.482- 483.
16. Hayder N, Abdelwaheda A, Kilania S, Ben Ammar R, Mahmoud A and Ghedirab K, Anti-

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

genotoxic and free-radical scavenging activities of extracts from (Tunisian) Myrtuscommunis, Mutat Res, 2004; 564: 89-95.

17. Dogan A, Investigations Myrtuscommunis L., plant's volatile oil yield, their physical-chemical properties and their compositions, Turkish: Ankara University, Agricultural Faculty Press, 1978, p. 678.

18. Rastogi R P and Mehrotra B N, Compendium of Indian Medicinal Plants (1970-1979), Central Drug ResearchInstitute Lucknow, Vol. 2, Publications and Information Directorate, CSIR, New Delhi, 1991, p. 478.

19. Giacomo M, Gas chromatographic-mass spectrometric investigation of the volatile components of myrtle berries (Myrtus communis L.), J Chromatogr, 1983;264: 304-311.

20. Jerkovic I, Radionic A and Borcic I, Comparative study of leaf, fruit and flower essential oils of Croatian Myrtus communis Linn. during a one year vegetative cycle, J. Essent Oil Res, 2002; 14 (4): 266-270.

21. Baitar ZI, Aljameul Mufradat Al-advia-wa- al-Aghzia, Vol. 1, Translated by CCRUM, New Delhi, 1999, pp. 42-47.

22. Ghani M N, Khazainul Advia, Sheikh Mohammad Bashir and Sons Publication, Urdu Bazar, Lahore, Vol. III, 1920, pp. 444-445.

23. Kabiruddin M, Makhzan-ul-Mufradat, Sheikh Mohammad Bashir and Sons, Lahore, Pakistan, 1951, pp. 47-48.

24. Hakeem M A, Bustanul Mufradat, Idara Tarraqui Urdu Publications, Lucknow, 1895, p. 278.

1. Trease W and Evans D, Pharmacognosy, 15th Edn, W.B. Saunders Comp Ltd., Toronto, 2006, p. 477.

2. Ali M and Ansari S H, Herbal drugs used as hair tonic, In: National seminar on the Use of Traditional Medicinal plants in skincare, CIMAP, Lucknow, November 25-26, 1994, p. 20.

3. Agarwal V S, Economic Plants of India, Kailash Prakashan, Calcutta, 1986, p. 251.

4. Elfellah M S, Akhter M H and Khan M T, Antihyperglycaemic effectof an extract of Myrtus communis in streptozotocin-induced diabetes in mice, J Ethnopharmacol, 1984, 11, 275-281.

5. Chalchat J, Garry R P and Michet A, Essential oils of myrtle of the mediterranean littoral, J Essent Oil Res, 1998; 10: 613-617.

6. Serce S, Ercisli S, Sengul M, Gunduz K and Orhan E, Antioxidant activities and fatty acid composition of wild grown myrtle (Myrtus communis L.) fruits, Phcog Mag, 2010; 6: 9-12.

7. Flaminia G, Cionia P, Morellia I, Maccionib S and Baldini, Phytochemical typologies in some populations of Myrtus communis L. on Caprione Promontory (East Liguria, Italy), Food Chem, 2004; 85: 599-604.

8. Rajkumar V. Evaluation of cytotoxic potential of Acorus calamus rhizome.Ethnobotanical Leaflets. 2009; 13 (6): 832-839.

9. Kumar SV, Sankar P and Varatharajan R. Anti-inflammatory activity of rootsof Achyranthes aspera. Pharmaceutical Biology. 2009; 47 (10): 973-975.

25. Sabu MC and Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Journal of Ethnopharmacology. 2002; 81 (2): 155- 160. Khare CP. Indian Medicinal Plants - An Illustrated Dictionary. Berlin, Springer, 2007.

26. Kirtikar KR, Basu BD. Indian medicinal plants. New Delhi, India. 2006.

27. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian medicinal plants.

CSIR New Delhi, India. 1956.

28. Venkatachalam T et al. Physicochemical and preliminary phytochemical studies on the Lantana Camara (L.) fruits. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3 (1): 52-54.

29. Kensa VM. Studies on phytochemical screening and antibacterial activities of Lantana camara Linn. Plant Sciences Feed. 2011; 1 (5): 74-79.

30. Kalita S et al. Phytochemical composition and in vitro hemolytic activity of Lantana camara L. (Verbenaceae) leaves. Pharmacologyonline. 2011; 1: 59-67.

31. Bhakta D, Ganjewala D. Effect of leaf positions on total phenolics, flavonoids and proantho-cyanidins content and antioxidant activities in Lantana camara (L). Journal of Scientific Research. 2009; 1 (2):

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

363-369.
32. Kalita S, Kumar G, Karthik L, Rao KV. A Review on Medicinal Properties of Lantana camara Linn. Research Journal of Pharmacy and Technology. 2012; 5(6): 711-5.
33. OECD. OECD Guidelines for the Testing of Chemicals No. 423: Acute Oral Toxicity— Acute Toxic Class Method.
34. Shoba FG, Thomas M. Study of antidiarrhoeal activity of fourmedicinal plants in castor-oil induced diarrhoea.J Ethnopharma-col. 2001; 76: 73-76.
35. Sisay M, Engidawork E, Shibeshi W. Evaluation of the antidiar-rheal activity of the leaf extracts ofMyrtus communis Linn (Myrtaceae) in mice model.BMC ComplementAltern Med. 2017; 17: 103.

1. Arzumand A, Saleh-e-In MM, Ahmed NU, Hashem MA, Bachar SC. Anti-diarrheal activity and acute toxicity of methanolic barkextract of Adenanthera pavoninaLinn (fabaceae) and its elemental composition. Turk J Pharm Sci. 2013; 10: 263-272.

1. Franca CS, Menezes FS, Costa LC, et al. Analgesic and antidiarrheal properties of Ocimum selloiessential oil in mice.Fitoterapia. 2008; 79: 569-573.
2. Ruwart MJ, Klepper MS, Rush BD. Clonidine delays small intestinal transit in the rat.J Pharmacol Exp Ther. 1980; 212: 487-490.
3. Atta AH, Mouneir SM. Evaluation of some medicinal plantextracts for antidiarrhoeal activity.Phytother Res. 2005; 19: 481-485.
a. Wang H, Peng D, Xie J. Ginseng leaf-stem: bioactive constituentsand pharmacological functions.Chin Med. 2009; 4: 20-28.
b. Agbor GA, L´eopold T, Jeanne NY. The antidiarrhoeal activity ofAlchornea cordifolialeaf extract.Phytother Res. 2004; 18: 873-876.
c. Petri WA, Miller M, Binder HJ, Levine MM, Dillingham R,Guerrant RL. Enteric infections, diarrhea, and their impacton function and development.J Clin Invest. 2008;118: 1277-1290.
d. Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal activity of80%methanolic leaf extract of Justicia schimperiana.Evid BasedComplement Alternat Med. 2018; 3: 37-42.
e. Niemegeers CJ, Awouters F, Janssen PA. The castor oil test inrats: an in vivo method to evaluate antipropulsive and antise-cretory activity of antidiarrheals? Drug Dev Res.1984; 4: 223-227.
f. Mascolo N, Izzo A, Barbato F, Capasso F. Inhibitors of nitricoxide synthetase prevent castor-oil-induced diarrhoea in the rat.Br J Pharmacol. 1993; 108: 861-864.

1. Brijesh S, Daswani P, Tetali P, Antia N, Birdi T. Studies onthe antidiarrhoeal activity ofAegle marmelosunripe fruit: vali-dating its traditional usage.BMC Complement Altern Med.2009; 9: 47-53.
2. Wood JD, Galligan JJ. Function of opioids in the enteric nervoussystem.Neurogastroenterol Motil. 2004; 16(suppl 2): 17-28.
3. Regnard C, Twycross R, Mihalyo M, Wilcock A. Loperamide.JPain Symptom Manage.

2011; 42: 319-323.

4. Chen W, Chung HH, Cheng JT. Opiate-induced constipation related to activation of small intestine opioidm2-receptors.WorldJ Gastroenterol. 2012; 18: 1391-1396.
5. Tadesse WT, Hailu AE, Gurmu AE, Mechesso AF. Experimentalassessment of antidiarrheal and antisecretory activity of 80%methanolic leaf extract of Zehneria scabra in mice.BMC Complement Altern Med. 2014; 14: 460-468.
6. Islam AMT, Uddin ME, Chowdhury MAU, Rahman MM, HabibMR, Rahman MA. In vivo antidiarrheal and cytotoxic potential ofdifferent fractions of Pandanus Foetidusleaves.Am J Biomed Sci.2013; 5: 208-216.
7. Lima JT, Almeida JR, Barbosa-Filho JM. Spasmolyticaction of diplotropin, a furanoflavan fromDiplotropis ferruginea Abdela9Benth, involves calcium blockade in Guinea-pig ileum.Z Natur-forsch B. 2005; 60: 1093-1100.
8. Atta AH, Mouneir SM. Antidiarrhoeal activity of some Egyp-tian medicinal plant extracts.J Ethnopharmacol. 2004; 92: 303-309.62.
9. Venkatesan N, Thiyagarajan V, Narayanan S. Anti-diarrhoeal potential ofAsparagus racemosuswild root extractsin laboratory animals.J Pharm Pharm Sci. 2005; 8: 39-46.
10. Belemtougri R, Constantin B, Cognard C, Raymond G, SawadogoL. Effects of two medicinal plantsPsidium guajavaL.(myrtaceae)andDiospyros mespiliformisL.(Ebenaceae) leaf extracts on ratskeletal muscle cells in primary culture.J Zhejiang Univ Sci B.2006; 7: 56-63.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

36. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemicalscreening and extraction:a review.Int Pharma Sci. 2011; 1: 98-106.
37. Jaganathan R, Ravinayagam V, Panchanadham S, Palanivelu S.Troxicological, biochemical and histopathological evaluation ofTridham, a siddha medicine in Wistaralbino rats.J BiochemTechnol. 2012 ;4: