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ANTI-DIARRHEAL AND ANTI-OXIDANT ASSESSMENT OF *MIMUSOPS ELENGI* LINN. UNRIPEN FRUIT EXTRACTS

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Keywords:

Mimusops Elengi, Anti-oxidant, Anti-diarrheal, Castor oil, Extracts

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ABSTRACT: The objective of the research was to investigate and evaluate anti diarrheal, antioxidant activity *Mimusops elengi* Linn. unripen fruit extracts. *Mimusops elengi* Linn. unripen fruits is considered as one of the best medicinal plants due to its several pharmacological uses mentioned in Unani as well as ethnomedicine. The various extracts of the plant (bark, fruit, leaves, seed, and flowers) have been disclosed to be cardiotonic, alexipharmic and stomachic, hypotensive, antibacterial, anthelmintic, anti gastric ulcers, teeth cleaner and renewable sources of energy. This review is an attempt to compile and document information on conventional uses and phytochemical properties of *Mimusops elengi* Linn. unripen fruits. The antidiarrhoeal activity of pet ether, chloroform and methanol extracts of *Mimusops elengi* unripen fruits were assessed using castor oil-induced diarrhea model in rats. Further, the effects of pet ether, chloroform, and methanol extract on gastrointestinal tract motility after charcoal meal dispensed were evaluated. Loperamide was taken as positive control. The plant extracts showed decisive and significant ($P<0.05$) thwarting action against castor oil-induced diarrhea in rats when tested at 200 mg/kg. The methanol extract showed decisive and significant ($P<0.001$) curtailed in gastrointestinal motility in charcoal meal test in rats. The results specify the possible anti-diarrheal effect of the plant extracts and confirmed the use of this herbal therapeutics as a non-specific treatment for diarrhea in folk medicine.

INTRODUCTION: *Mimusops genus* belongs to a family Sapotaceae that comes under sub kingdom Vascular plants. *M. elengi* is evergreen medium to large-sized tree. The tree is native to India, Sri Lanka, the Andaman Islands, Myanmar, and Indo-China, but is commonly planted as an ornamental tree throughout the tropics, also in Africa. Different parts of this plant are utilized in the traditional system of medicine for treatment of different ailments [1]. In Ayurveda, the bark, flowers, fruit and seeds are of great value for treating various diseases.

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The diseases or illnesses such as cardiotonic, alexipharmic, stomachic, astringent cooling, anthelmintic, tonic, and febrifuge properties and is also helpful in alleviating kapha and pitta doshas are treated by using different parts of *M. elengi* [2].The bark and fruits of this plant are used in the medication and treatment of diarrhea and dysentery and decoction of the bark is used as a gargling agent [3]. The leaves are familiar with analgesic and antipyretic [4]. *M. elengi* also utilized in sanitation dermal wounds, anti-ulcer effects and boosted the fertility in women.

As the literature suggested that no investigation is yet carried out regarding antioxidant and antidiarrhoeal activity of unripe fruits of *Mimusops elengi* Linn. The present study is aimed to evaluate the antidiarhoeal and antioxidant activity of *Mimosops elengi* unripe fruit extracts.

MATERIALS AND METHODS:

Plant Material: Fresh raw fruits of *Mimusops elengi* unripen were collected from Salipur, Cuttack, Odisha, India which was identified and authenticated by Associate professor Dr. Gita Dash. The voucher specimen was given the no. 001/18.

Preparation of Extract: The air-dried powdered unripe fruits were loaded into Soxhlet apparatus and were subjected to extraction for about 72 h with petroleum ether (60-80 ºC), chloroform and methanol successively. The solvents were distilled and evaporated off after extraction, and the extracts were concentrated and reduced under reduced pressure. The extracts were kept in refrigerator until tested [6]. The four extracts were then dissolved subjected to phytochemical analysis.

For the pharmacological tests, the extracts were dissolved in 1% Tween-80 in normal saline solution to prepare 200 mg/kg concentrations [5]. The chemical constituents of the extracts were identified by qualitative chemical tests and further confirmed by GCMS study for the presence of alkaloids, carbohydrates, sterols, and flavonoids.

***In-vitro* Antioxidant Assessment:**

Superoxide Scavenging Activity: Superoxide scavenging action of the plant extract was dictated by McCord and Fridovich's method, 1969, which relies on light-evoked superoxide generation by riboflavin and the relating reduction of nitroblue tetrazolium. The plant extracts of 0.1 ml of different concentrations and 0.1 ml of 6 µM ethylenediamine tetraacetic acid comprises of NaCN, 0.1 ml of 50 µM nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and last volume was made up to 3 ml using phosphate buffer.

Then the assay tubes were uniformly illuminated with incandescent light (40 Watts) for 15 min and thereafter the optical densities were estimated at 560 nm. A control was prepared using 0.1 ml of individual vehicle in the place of plant extract/ ascorbic acid. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of control and experimental tubes [6, 7].

Percentage inhibition was determined by using the following equation:

$$\% \text{ Inhibition} = [(A_0-A_1) / A_0 \times 100]$$

Where; A_0 is absorbance of the control and A_1 is absorbance of test sample. IC_{50} value was calculated from the values obtained for % Inhibition.

Hydroxyl Radical Scavenging Activity: Hydroxyl radical scavenging activity was estimated by studying the competition between deoxyribose and the extracts for hydroxyl radicals created from the $Fe^{2+}/EDTA/H_2O_2$ framework reaction (Fenton reaction). The hydroxyl radical assaults deoxyribose, which in the long run outcomes in the development of thiobarbituric acid reacting substances (TBARS) (Elizabeth and Rao, 1990). Fenton reaction mixture comprises of 200 µl of 10 mM ferrous sulphate ($FeSO_4.\ 7\ H_2O$), 200 µl of 10 mM EDTA and 200 µl of 10 mM 2- deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 µl of plant extracts. Thereafter, 200 µl of 10 mM H_2O_2 was added before the incubation at 37 ºC for 4 h.

Then, 1 ml of this Fenton reaction mix was employed with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20% acetic acid. The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100 ºC for 1 hour. After the blend had been cooled, 5 ml of 15:1 v/v butanol-pyridine mix was added and included. Following energetic shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was estimated at 532 nm. A control was assembled using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The rate of restraint or percentage inhibition of hydroxyl radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes as calculated for hydroxyl radical assay [8, 9].

DPPH Radical Scavenging Activity: Free radical scavenging or rummaging action was predispose or inclined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical with a slight alteration. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extracts at various concentrations were mixed and blended. The mixture of the blend was shaken energetically, vigorously and allowed

to reach a constant state at room temperature for 30 min. Decolorization of DPPH was resolute by measuring the absorbance at 517 nm. A control was arranged by using 0.1 ml of respective vehicles in the place of plant extract/ascorbic acid [10, 11].

The percentage restraint or inhibition action was figured as $[(A_0-A_1)/A_0] \times 100$, where A_0 was the absorbance of the control, and A_1 was the absorbance of the plant extract/ ascorbic acid. IC_{50} values (*i.e.* concentration of a sample, which is required to scavenge exact 50% of free radicals) were also calculated from the data obtained.

Animals: Inbreed Albino Wistar rats of either sex weighing somewhere between 200 and 260 g were utilized for the study. They were housed in polyacrylic cages and fed with standard rodent pellet diet and given water *ad libitum*. The animals were housed under standard research facility ecological condition for an acclimatization time of 14 days preceding play out the analyses. Every test convention was endorsed by the institutional creature morals council before the direct of investigations (27/IAEC/IPT/17).

Castor Oil Induced Diarrhea: Rats were divided into five groups (n = 6) and, fasted for 18 h and water was provided ad libitum. The methanol (200 mg/kg, p.o.), chloroform extract (200 mg/kg, p.o.) and pet ether extracts of *Mimusops elengi* unripen fruits were administered orally to the first three groups of rats. One group received 10 ml/kg 0.5% v/v aqueous Tween 80 and served as a negative control. Another group received the standard drug loperamide (3 mg/kg, p.o.) as a positive control. After 1 h of treatment, all the animals were challenged with 1 ml of castor oil orally, by gavages and watched for consistency of fecal material [12, 13].

The recurrence or frequency of defecation was noted in transparent plastic dishes placed beneath the individual rat cages up to 4 h, and the weight of fecal matter was determined. The % protection was determined using the formula % protection= (mean wt. of stool in control mean wt. in treated) X 100/ mean wt. of stool in control.

Small Intestinal Transit: Rats were divided into five groups (n = 6) and fasted for 18 h before the experiment. Each animal was given or administered with 1 ml of charcoal meal orally (5% activated charcoal in 5% acacia) followed by oral administration of methanol (200 mg/kg, p.o.), chloroform extract (200 mg/kg, p.o.) and pet ether extracts to the first three groups or sets of animals. The fourth group was treated with 0.5% v/v aqueous Tween 80 (10 ml/kg, p.o.) and served as a negative control. The fifth group received atropine (0.1 mg/kg, i.p.), as the positive control. Thirty minutes later, each animal was killed and the intestinal distance moved by the charcoal meal from the pylorus to caecum was measured and expressed as the percentage of distance moved [14, 15].

Statistical Analysis: The experimental results were analysed using the Statistical Package for the Graphpad Prism results are expressed as a mean ± standard error of the mean (SEM), and statistical analyses were carried out by employing one-way analysis of variance (ANOVA), followed by Tukey T-test to compare results with controls. In all cases, statistical significance was set at $p<0.05$.

RESULTS: The results of the preliminary phytochemical screening of methanol and aqueous extracts of *Mimusops elengi* unripened fruits flower have been presented in **Table 1.**

TABLE 1: PHYTOCHEMICAL SCREENING OF *MIMUSOPS ELENGI* UNRIPEN FRUITS

Experiment	Petroleum ether extract	Chloroform extract	Methanol extract
Test for Carbohydrates			
Molisch's Test	-	-	+
Fehling's Test	-	-	+
Benedict's Test	-	-	+
Barfoed's Test	-	-	+
Bial's Test	-	-	-
Aniline Acetate Test	-	-	-
Cobalt-chloride Test	-	-	+
Tollen's Phloroglucinol Test	-	-	-
Selwinoff's Test	-	-	-
Inversion Test	-	-	+
Iodine Test	-	-	-

Test for Gums and Mucilages			
Swelling Test	-	-	-
Test for Proteins and Amino Acid			
Ninhydrin Test	-	-	+
Biuret Test	-	-	-
Tannic Acid Test	-	-	++
Heavy Metal Test	-	-	-
Million's Test	-	-	-
Xanthoprotein Test	-	-	-
Experiment	**Petroleum ether extract**	**Chloroform extract**	**Methanol extract**
Test for Fixed Oils and Fats			
Spot Test	+++	-	-
Saponification Test	-	-	+
Test for Phytosterols			
Libermann's Test	++	-	+
Salkowski's Test	++	-	-
Libermann-Burchard's Test	++	+++	++
Test for Glycosides			
Baljet's Test	-	-	++
Legal's Test	-	-	+
Borntrager Test	-	-	-
Modified Borntrager Test	-	-	-
Cyanogenetic Glycoside Test	-	-	-
Raymond's Test	-	-	-
Tollen's Test	-	-	-
Xanthydrol Test	-	-	-
Antimony Trichloride Test	-	-	++
Keddie's Test	-	-	-
Test for Saponins			
Foam Test	-	-	+
Haemolytic Test	-	-	++
Test for Flavonoids			
Ferric Chloride Test	-	-	++
Shinoda Test	-	-	++
Lead Acetate Test	-	-	++
Fluorescence Test	-	+	++
Action of Alkali and Acid	-	-	+++
Test for Tannins and Phenolic Compounds			
Ferric Chloride Test	-	-	++
Test with Heavy Metals	-	-	++
Nitric Acid Test	-	-	++
Goldbeater Skin Test	-	-	-
Experiment	**Petroleum ether extract**	**Chloroform extract**	**Methanol extract**
Gelatin Test	-	-	++
Phenazone Test	-	-	++
Catechin Test	-	-	++
Chlorgenic Acid Test	-	-	++
Vanillin-HCl Test	-	-	++
Test for Alkaloids			
Mayer's Test	-	++	-
Dragendorff's Test	-	-	-
Wagner's Test	-	++	-
Hager's Test	-	-	-
Sonneuschein's Test	-	-	-
Scheibler's Test	-	-	-
Tannic Acid Test	-	-	-

All the three extracts were screened for phyto-chemical investigation by different phytochemical tests to check the presence or absence of a group of phytochemical constituents. These phytochemical tests showed the presence of proteins, carbohy-drates, alkaloids, saponins, tannins, flavonoids, steroids, triterpenoids, *etc*.

Petroleum ether extract gave positive tests for fats and phytosterol; chloroform extract showed positive tests for phytosterol, glycoside, flavonoids, and alkaloids; methanol extracts were found to contain carbohydrate, protein, phytosterol, glycol-side, saponin, flavonoids and polyphenols. The phytochemical like polyphenolic compound present

in the methanol extracts of *Mimusops elengi* Linn. May be responsible for the antioxidant and anti-diarrheal activity.

***In-vitro* Antioxidant Activity:** Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA. Therefore, studying the scavenging activity of plant extracts/compounds on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity. In the present study, the petroleum ether, chloroform and methanol extracts of *Mimusops elengi* were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results were given in **Fig. 1.** The mean IC_{50} values for superoxide radicals of petroleum ether, chloroform and methanol extracts of *Mimusops elengi* were found to be 520.629µg, 375.111µg, and 310.81µg respectively. The mean IC_{50} value of ascorbic acid was found to be 255.6 µg were given in **Fig. 2.** The petroleum ether, chloroform, and methanol extracts of *Mimusops elengi* were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given in **Fig. 3.** The mean IC_{50} values for hydroxyl radical of petroleum ether, benzene, chloroform and methanol extracts of *Mimusops elengi* were found to be 484.379 µg, 340.727 µg, 342.727 µg, and 259.322 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 183. 384 µg given in **Fig. 4.** The petroleum ether, chloroform and methanol extracts of *Mimusops elengi* were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given in **Fig. 5.** The mean IC_{50} values for DPPH radical of petroleum ether, chloroform and methanol extracts of *Mimusops elengi* were found to be 340.193 µg, 185.726 µg, and 120.00 µg respectively. The mean IC_{50} value of ascorbic acid was found to be 25.68µg given in **Fig. 6.** Among the four types of *Mimusops elengi* extracts, the methanol extract showed better scavenging activity The order of scavenging activity was in the following manner: ascorbic acid > methanolic extract > chloroform > petroleum ether.

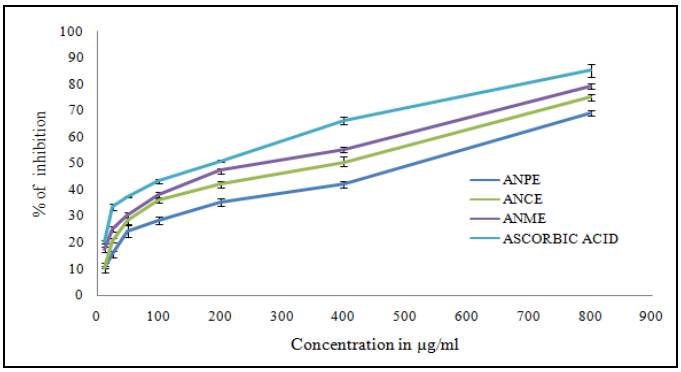

FIG. 1: DETERMINATION OF SUPEROXIDECAL SCAVENGING ACTIVITY

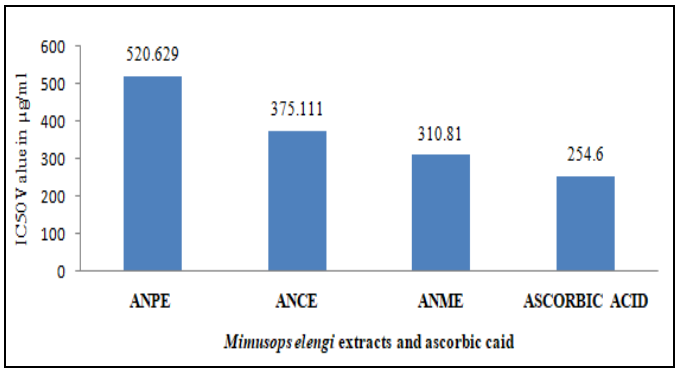

FIG. 2: IC_{50} OF *MIMUSOPS ELENGI* EXTRACTS AGAINST SUPEROXIDE REDICAL

MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract

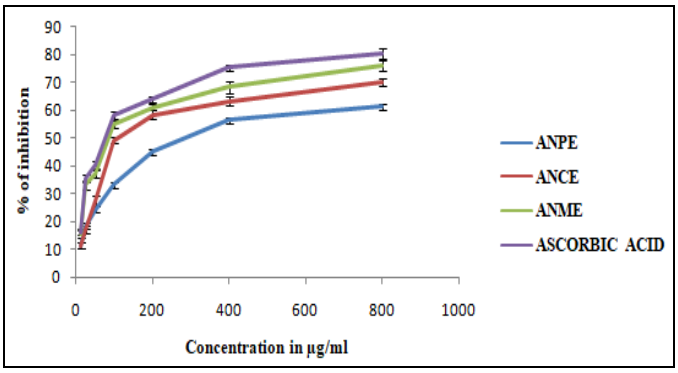

FIG. 3: DETERMINATION OF HYDROXYL RADICAL SCAVENGING ACTIVITY

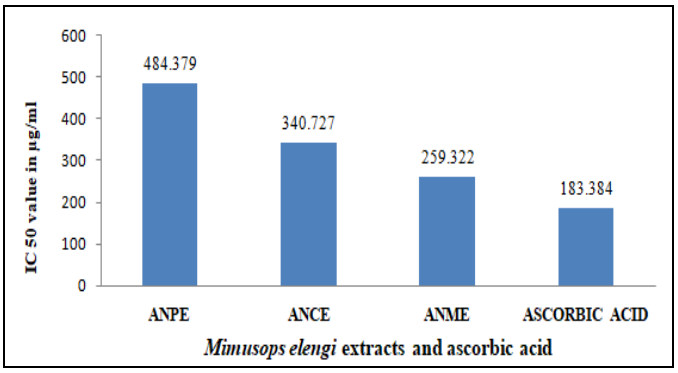

FIG. 4: IC_{50} OF *MENUSOPS ELENGI* EXTRACTS AGAINST HYDROXYL

MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract

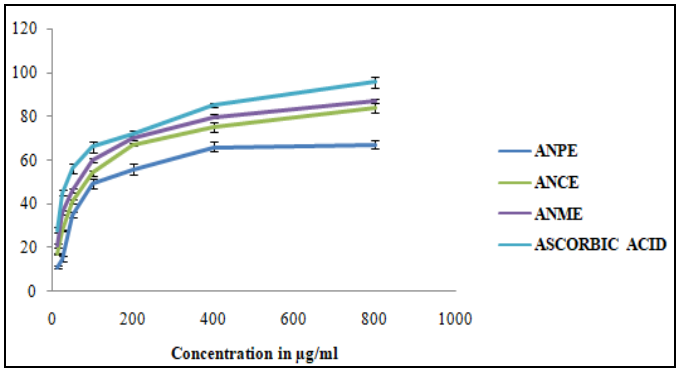

FIG. 5: DETERMINATION OF DPPH RADICAL SCAVENGING ACTICITY

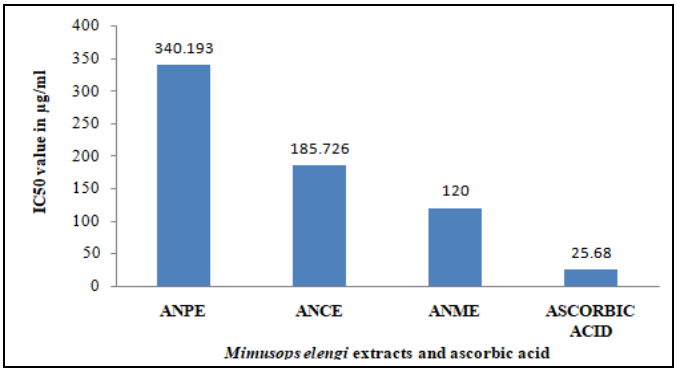

FIG. 6: IC_{50} OF *MIMUSOPS ELENGI* EXTRACTS AGAINST DPPH RADICAL

MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract

Castor Oil Induced Diarrhoea: Administration of castor oil produced characteristic semi-solid diarrhea dropping in 18 h starved rats of the control group during the 4 h observation period. The methanol extract dose of 200 mg/kg showed significant ($P<0.05$) reduction (2.16 ± 0.30 *) in the number of defecations over four hours when compared to that of untreated control rats (5.5 ± 0.70); the activity was similar to that of loperamide 3 mg/kg (1.83 ± 0.40*), the standard anti-diarrhoeal agent. The chloroform and pet ether extract doses of 200 mg/kg showed (4.33±0.33 and 4.83±1.25) reduction of defecations over four hours when compared to that of untreated control rats shown in **Fig. 7.**

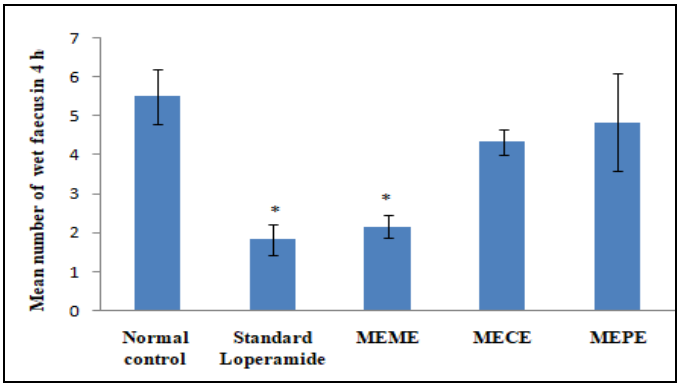

FIG. 7: EFFECT OF *MIMUSOPS ELENGI* EXTRACTS ON CASTOR OIL INDUCED DIARRHOEA IN RATS. MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract. Results are expressed as a mean ± standard error of the mean (SEM), and statistical analyses were carried out by employing a one-way analysis of variance (ANOVA), followed by Tukey T-test to compare results with controls. In all cases, statistical significance was set at $p<0.05$

The mean wet of feces produced by the loperamide, methanol, chloroform, and pet ether extract was found to be 13.25±0.68*, 16.83±0.30*, 53.33±2.21 and 60.5±1.87 respectively shown in **Fig. 8**.

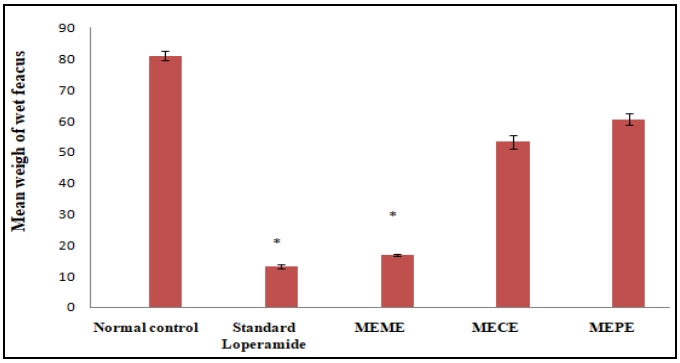

FIG. 8: EFFECT OF *MIMUSOPS ELENGI* EXTRACTS ON CASTOR OIL INDUCED DIARRHOEA. MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract. Statistical significance test with control was done by Tukey Kramer multiple comparison test Anova tests. *P < 0.05.

The loperamide, methanol, chloroform, and pet ether extract showed 83.65%, 79.24%, 34.22% & 25.38% protection against castor oil-induced diarrhea at four hours respectively when compared to untreated control group shown in **Fig. 9**. As *Mimusops elengi* extract remove successfully restrained the castor oil actuated diarrhea, it very well may be expected that the antidiarrhoeal activity was applied by antisecretory mechanism [16, 17].

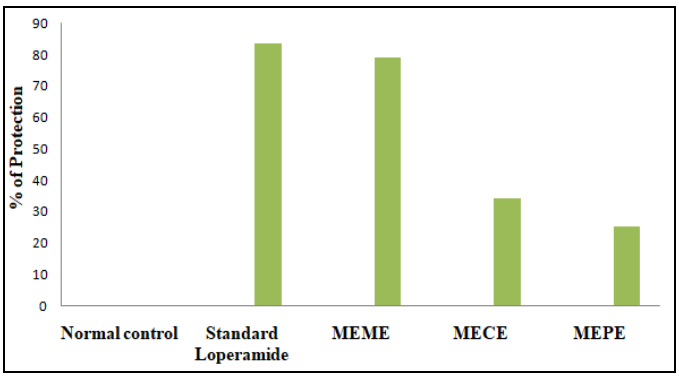

FIG. 9: EFFECT OF *MIMUSOPS ELENGI* EXTRACTS ON CASTOR OIL INDUCED DIARRHOEA. MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract.

Effect of *Mimusops elengi* unripen fruits extracts on gastro intestinal transit in rats is shown in **Fig. 10.** The methanol extract (200 mg/kg), decreased propulsion (25.16 ± 1.27***) of the charcoal meal through the gastrointestinal tract compared to control (0.5% Tween 80) significantly (73.16%). The chloroform and pet ether extract showed decreased propulsion of the charcoal (55.16 ± 1.42 and 62.33 ± 1.68), respectively, in comparison to control group. The percentage of inhibition of standard drugs, methanol, chloroform, and pet ether extract was 65.60%, 46.92%, 24.60%, and 14.80%, respectively.

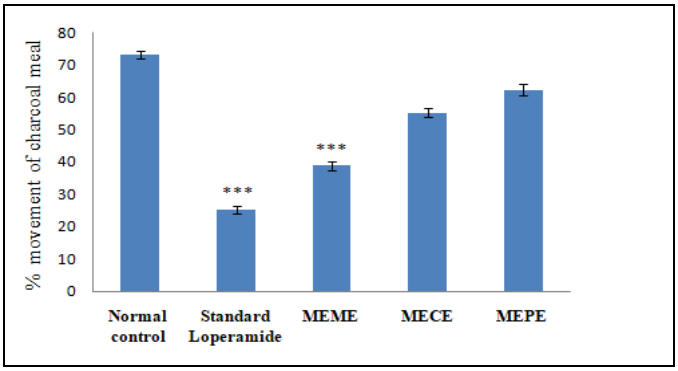

FIG. 10: EFFECT OF *MIMUSOPS ELENGI* UNRIPENS FRUITS EXTRACTS ON GASTROINTESTINAL TRANSIT IN RATS. MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract.Results are expressed as a mean ± standard error of the mean (SEM), and statistical analyses were carried out by employing one-way analysis of variance (ANOVA), followed by the Tukey T-test to compare results with controls. In all cases, statistical significance was set at $p<0.05$.

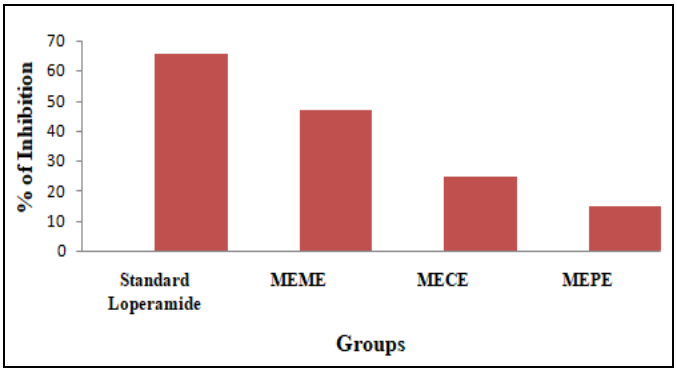

FIG. 11: EFFECT OF *MIMUSOPS ELENGI* UNRIPEN FRUITS EXTRACTS ON GASTROINTESTINAL TRANSIT IN RATS. MEME: *Mimusops elengi* methanol extract; MECE: *Mimusops elengi* chloroform extract; MEPE: *Mimusops elengi* Pet-ether extract.

A similar reduction in the gastrointestinal transit of charcoal meal in the rat was achieved with the intra-peritoneal injection of atropine sulphate (0.1 mg/kg) shown in **Fig. 11**. GI motility depicts the contraction of the muscles that blend and propel contents in the gastrointestinal tract. Charcoal meal test in rodents is a technique used to study the impact of medication on the motility of intestine. In present study *Mimusops elengi* methanol extract was observed to be the inhibitor of intestinal motility [18].

CONCLUSION: The phytochemical analysis of the extracts demonstrated the presence of alkaloids, saponins, flavonoids, tannin, sterols and/or terpenes and sugars. These constituents may in charge or responsible for the antidiarrhoeal activity of *Mimusops elengi* unripe fruits.

The antidiarrhoeal activity of tannin or polyphenols may ascribe to their ability to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition. *In-vitro* and *in-vivo* experiments have shown that tannin or polyphenol are able to inhibit the intestinal secretory response, induced by prostaglandins E2.

The antioxidant activity may also be ascribed to the presence of a polyphenolic compound present in the methanol fruit extract. The results indicate that the methanol extract of *Mimusops elengi* unripe fruits possess significant anti-diarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion.

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CONFLICTS OF INTEREST: Nil

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