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Research Article

## A Comparative Study of Analgesic, Antidiarrhoeal and Antimicrobial Activities of Methanol and Acetone Extracts of Fruits Peels of *Limonia acidissima* L. (Rutaceae)

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### ABSTRACT

**Objectives:** Present study was designed to evaluate Analgesic, Antidiarrhoeal and Antimicrobial activities of methanol and acetone extracts of fruit peels of *Limonia acidissima* L. by different methods.

**Method:** The analgesic activity of the samples was studied using acetic acid- induced writhing model in mice. Castor oil-induced antidiarrheal activity was observed by Thomas method and antimicrobial activity was monitored by disc diffusion method.

**Results:** *Limonia acidissima* L. inhibited 60.53 % and 59.65 % writhing of methanol as well as acetone fruit peels extracts, respectively, compared to standard drug Diclofenac Na inhibited 78.07 % writhing. At higher dose (500 mg/kg) of the methanol and acetone fruit peels extracts, significant inhibition 47.13 and 44.83 % of characteristic diarrhoeal feces was observed, respectively, as well as at lower dose (250 mg/kg) of the both extracts, inhibition 34.45 and 35.63 %. Mentionable on average 12mm zone of inhibition was observed of both extract at 250µg/disc and 500µg/disc compare to zone of inhibition 36mm of ciprofloxacin at 50µg/disc.

**Conclusion:** From the above results, it will be very much possible source for an isolating lead compound for curing the numerous disorders.

**Keywords:** *Limonia acidissima* L. Fruit peels, Diclofenac sodium, Loperamide, Ciprofloxacin.

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### INTRODUCTION

The use of medicinal plants as a source for abatement from ailment can be marked back over five millenary to written documents of the early civility in China, India and the Near east. From that time it is used abundantly for the curing purpose of the mankind.<sup>1-2</sup>

*Limonia acidissima* L. is the family of Rutaceae (Citrus family) which belongs to the monotypic genus *Limonia*, confined to India, Pakistan, Sri Lanka and Southeast Asia<sup>3</sup>. It is also known as woodapple, elephant-apple, curd fruit, kath bel as well askaitha. This plant parts are used as a medicine for the treatment of several disorders<sup>4</sup>. Wood apple is an erect, slow-growing tree with a few upward-reaching branches bending outward near the summit. The bark is ridged, fissured and scaly. The deciduous, alternate leaves, 3 to 5 in long, dark-green, leathery, often minutely toothed. Yellowish green flowers, tinged with red, 1/2 in across, are

borne in small, loose, terminal or lateral panicles. The tree is mostly known for its hard woody fruit, size of a tennis ball, round to oval in shape. The pulp is brown, mealy, odorous, resinous, astringent, acid or sweetish.<sup>5</sup> Especially it is used for treating indigestion, flatulence, diarrhoea, dysentery and haemorrhoids. The bark is chewed with that of *Barringtonia* and applied on venomous wounds<sup>6</sup>.

NSAIDs (Non-steroidal anti-inflammatory drugs) are widely used as analgesic drugs which are used as relieving pain as well as inflammation. Analgesic are the drugs which relieve pain selectively without blocking the conduction of nerve impulses, noticeably changing sensory sensation, whether touching consciousness<sup>7,8</sup>. Analgesics minimize the levels of chemical mediators (prostaglandins) produced during inflammation, relieving symptoms of pain, swelling as well as redness. They inhibit the enzyme cyclo-oxygenase (COX 2) that is integral in the synthesis of prostaglandins. Through infection, the effect of prostaglandins on the

hypothalamus resulting increasing body temperature. They are not only inhibiting local prostaglandin production, but also whole the body<sup>9</sup>. Diarrhoeal disease has long been perceived as a leading cause of morbidity and mortality; paramount cause of sickness as well as death among young children especially in developing countries<sup>10</sup>. Diarrhoea is distinguished by increased frequency of bowel movement, watery stool as well as abdominal pain. Miscellaneous national and international organizations are trying to control this disease but the rate of incidence is still high, approximately 7.1 million per year. A lot of synthetic chemicals are available for the treatment of diarrhoea but they have some major side effects<sup>11,12</sup>.

Medicinal plants represent a rich origin of antimicrobial agents. Plants are used medicinally in different countries as well as are a source of many potent and powerful drugs<sup>13</sup>. Antimicrobial compounds that play an essential role in the natural advocacy of all kinds of living organisms<sup>14</sup>. The antimicrobial compounds from natural sources may be inhibited bacteria by a different mechanism than the presently used antibiotics and may have clinical value in treatment of resistant microbial strains<sup>15</sup>.

Development of newer and more powerful drugs with lesser side effects, plants could be the best choice.

## MATERIALS AND METHODS:

### Chemicals and reagents

Diclofenac sodium, loperamide, ciprofloxacin, acetic acid and castor oil were used.

### Plant Materials

The peels parts of the fruit of *Limonia acidissima* L. were collected from near Jahangir Nagar University fields, Dhaka, Bangladesh. The identification of the plant material was confirmed by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and also by the authorities of Botanical Garden, Mirpur, Dhaka.

### Drying and grinding

The collected fruit peels were separated from undesirable materials. Then these were dried in for one week in the sunlight and these were cutting into small pieces. The fruit peels were converted into coarse powder by using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

### Preparation of methanol and acetone extract

At first, two clean flat flat-bottomed glass containers was taken and added about 400 and 450gm of powdered sample into the container, respectively. Then 1500 ml of 90% methanol and 1800ml acetone were added into the two containers as well as soaked the powder into the methanol and acetone, respectively. Afterwards, containers were sealed with their contents and kept for a period of 10 days accompanying occasional shaking and stirring. After that, the coarse parts of the fruits were separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, these were filtered through whatman filter paper. Then the filtrate was kept in Rotary evaporator machine which separates solvent and desirable crude extracts was obtained.

### Experimental animals

Swice albino mice (22-25g) were purchased from Jahangir Nagar University, Dhaka, Bangladesh and their ages five to six weeks and were housed in animals cages under standard

environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University, Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

### Test Microorganisms

Five pathogenic bacterial strains were used to evaluate Antimicrobial activity. Three of them were Gram negative (*Klebsiella Oxytoca*, *Vibrio metschnikovii*, *Escherichia coli*) and two was gram positive (*Bacillus subtilis*, *Staphylococcus aureus*). All of the bacterial strains were collected from Microbiology Lab of Department of Pharmacy, Dhaka University, Dhaka, Bangladesh.

### Analgesic activity

For analgesic test all mice were divided into six groups. Each group comprises of 4 mice. Control group (received 0.5% methyl cellulose, per oral), Standard Group (received Diclofenac-Na10mg/ kg intraperitoneally), group III and IV were treated with methanolic fruit peels extracts of *Limonia acidissima* L. at the doses of 250 and 500 mg per kg of body weight, respectively and), group V and VI were treated with acitonic fruit peels extracts of *Limonia acidissima* L. at the doses of 250 and 500 mg per kg of body weight, respectively. The analgesic activity of the samples was studied using acetic acid- induced writhing model in mice. Test samples and vehicle were administered orally 30 mins before intraperitoneal administration 10ml/kg of 0.7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 minutes before the acetic acid injection, the mice were observed for specific contraction of body referred to as "writhing" for the next 10minutes [16]. Percentageprotection of acetic acid induced writhing was calculated by the formula. Percentage protection =  $(Wc - Wt) / Wc \times 100$ ; Where, wc is the mean values of control group and Wt is the mean values of treated group.

### Castor oil-induced diarrhoea

24 mice were allowed to fast for 18 h and divided into six groups of four animals each. All groups received castor oil at a dose of 1 ml/animal orally (p.o.). 30 min after castor oil administration, group I (control group) received vehicle (1% CMC in distilled water), Group III and Group IV orally received the methanol extract at 250 mg/kg and 500 mg/kg doses, respectively and ), Group V and Group VI orally received the acitone extract at 250 mg/kg and 500 mg/kg doses, respectively. Group II received the reference drug, loperamide (3 mg/kg p.o.). Then the animals were placed separately in cages with filter papers underneath, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h and the characteristic diarrhoeal droppings were recorded<sup>17</sup>.

### Test of antimicrobial activity by disc diffusion method

In this method-measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration ( $\mu\text{g/ml}$ ). Then sterile materiel filter paper discs are impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried are used as positive and negative control, respectively. These discs are then placed in petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening . The plates are then kept at 40°C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator (37° C) for

12-18 hour to allow the growth of the microorganisms. If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The Antimicrobial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter. The experiments are

carried out three times and the mean of the reading are recorded <sup>18</sup>.

### Statistical Analysis

The results are presented as Mean  $\pm$  SEM. Data were analyzed by one-way ANOVA followed by Dunnett's test and P values <0.001 were considered statistically significant.

## RESULT AND DISCUSSION

**Table 1** Results of Analgesic effect of *Limonia acidissima* L. fruit peels of methanol(ME) and acetone(AE) extracts on acetic acid-induced writhing in mice.

Treatment	Writhing counting( Mean $\pm$ SEM)	% of Inhibition
Control	28.5 $\pm$ 0.77	-
Standard (Diclofenac Na)	6.25 $\pm$ 0.227***	78.07%
ME 250mg	12 $\pm$ 0.37***	57.89%
ME 500mg	11.25 $\pm$ 0.42***	60.53%
AE 250mg	12.5 $\pm$ 0.77***	56.14%
AE 500mg	11.5 $\pm$ 1.18***	59.65%

Values are presented as Mean  $\pm$  SEM (n = 4), P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett's test). \*\*\*Indicates the significance of the result.

*Limonia acidissima* L. inhibited 60.53 % and 59.65 % writhing of methanol as well as acetone fruit peels extracts, respectively, compared to standard drug Diclofenac Na inhibited 78.07 % writhing. Here strong prominent effects were observed with both extracts group (500 mg/kg) and this effect is like that of standard group (Table 1). Pain is not always remediable, however there are various ways to medicate it. There are drug treatments, with along pain relievers <sup>19</sup>. The ability to detect noxious stimuli is essential

to an organism's survival and wellbeing. The explanation of molecules as well as cell types which underlie normal (acute) pain sensation is key to understanding the mechanisms basic pain hypersensitivity <sup>20</sup>. At present, plentiful pain therapies are either inadequate or dangerous side effects. Chronic pain results not only from the corporal insult but also from a combination of physical, emotional, psychological, as well as social abnormalities <sup>21</sup>.

**Table 2:** Effect of methanol(ME) and acetone(AE) extracts of the fruit peels of *Limonia acidissima* L. on castor oil-induced diarrhoea in mice.

Treatment	No. of faecal droppings in 4h (Mean $\pm$ SEM)	% Inhibition of defaecation
Control	21.75 $\pm$ 1.18	-
Standard (Loperamide)	7.0 $\pm$ 0.37***	67.82
ME 250mg	14.25 $\pm$ 0.77***	34.45
ME 500mg	11.50 $\pm$ 0.86***	47.13
AE 250mg	14.0 $\pm$ 0.97***	35.63
AE 500mg	12.0 $\pm$ 0.37***	44.83

Values are presented as Mean  $\pm$  SEM (n = 4), P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett's test). \*\*\*Indicates the significance of the result.

The extract at the doses of 250 and 500 mg/kg, produced a dose dependent reduce in the number of faecal matters passed by the mice in castor oil-induced diarrhoeal model (Table 2). At higher dose (500 mg/kg) of the methanol and acetone fruit peels extracts, significant inhibition 47.13 and 44.83 % of characteristic diarrhoeal feces was observed, respectively, as well as at lower dose (250 mg/kg) of the both extract, inhibition 34.45 and 35.63 % of diarrhoeal

feces was observed, respectively. The active component of castor oil is the ricinoleic acid, which is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. As a result, delay diarrhoea induced with castor oil <sup>22</sup>.

**Table 3:** In vitro Antimicrobial activity of *Limonia acidissima* L.on methanol(ME) and acetone(AE) fruit peels extracts.

Bacterial Strains	Type of bacteria	Diameter of zone of inhibition					
		Blank	Ciprofloxacin(50µg /disc)	ME(500 µg/disc)	ME (250 µg/disc)	AC(250 µg/disc)	AC(500 µg/disc)
<i>Klebsiella Oxytoca</i>	Gram(-)	-	36mm	12mm	9mm	11mm	7mm
<i>Vibrio metschnikovii</i>	Gram(-)	-	41mm	11mm	16mm	13mm	14mm
<i>Escherichia coli</i>	Gram(-)	-	38mm	15mm	19mm	14mm	17mm
<i>Bacillus subtilis</i>	Gram(+)	-	39mm	14mm	14mm	10mm	16mm
<i>Staphylococcus aureus</i>	Gram(+)	-	42mm	14mm	17mm	15mm	14mm

Gram (-):-Gram Negative Bacteria, Gram (+):-Gram Positive Bacteria (-):- No inhibition

The antimicrobial effects of methanol and acetone fruit peels extracts against different test organisms are shown (Table 3). Both extracts were showed moderate inhibitory activity against all of these organisms.

## CONCLUSION

This study on both extracts of *Limonia acidissima* L. has exhibited that this plant fruit peels has significant analgesic as well as antidiarrhoeal and moderate antimicrobial properties.

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**Compliance with Ethical Standards:** The handling and use of animals were in accordance with the National Institute for Health Guide for the Care and Use of Laboratory Animals. Our study was approved by a Research Ethics Committee for animal house of department of pharmacy, Faculty of Allied Health Sciences, Daffodil International University.

**Conflict of Interest:** The authors declared that they have no conflict of interest.

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