



## Original Article

# Anti-Arthritic and Anti-Microbial Effects of the Fruits of *Grewia Subinaequalis* D.C.

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The aim of the present study is to carry out the *invitro* antiarthritic activity of the alcoholic and aqueous extracts of the fruits of *Grewia subinaequalis* and to evaluate the antimicrobial activity of the ethanolic extract of the fruit. The antiarthritic activity was carried out using protein denaturation method. The fruit extract of the plant produced remarkable antiarthritic activity and the activity produced was comparable to activity produced by acetyl salicylic acid which was used as the reference standard during the evaluation. The anti bacterial activity of the ethanolic extract was evaluated by agar well diffusion method and the result indicates the potent antimicrobial action of the fruit.

**Keywords:** *Grewia subinaequalis*, Arthritis, Protein denaturation, *Escherichia coli* and *Lactobacillus acidophilus*

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## 1. INTRODUCTION

Arthritis can be clearly say as an informal way of referring to joint pain and is most common among women, which occur frequently as people get older.

Arthritis condition can also be defined as an auto immune disorder which is associated with pain and swelling<sup>1</sup>. Generally arthritis is an inflammation of synovial joint due to immune mediated response. But the usage of anti-inflammatory drugs in treating arthritis is not advisable all the times, because it does not suppress T-cell and B-cell

mediated response<sup>2</sup>. Even though there are many modern drugs to treat such disorder, their prolonged usage may cause severe side effects. Hence there is a strong desire to develop new therapeutic agents with minimum side effects.

*Grewia subinaequalis* from Tiliaceae family is a food plant and can also be considered as an herbal medicine for the treatment of various diseases. The plant is commonly found in the greater part of India like; Himalaya, Punjab, Bengal, Bihar, Orissa, Tamilnadu, Karnataka, Andrapradesh and Kerala<sup>3</sup>. The fruit of the plant is a fleshy fibrous drupe and grayish purple in colour at maturity. On the surface of the fruits black coloured circular depressed spots with large and small stellate covering trichomes can be seen. *Grewia subinaequalis* is particularly used as a medicinal plant by the traditional users since ancient times. But there are not much scientific data regarding the usage of the plant. Ayurveda and other traditional system of medicine support the use of the plant as an antidiabetic, antileprotic, antiinflammatory and also in treating rheumatoid arthritis<sup>4,5</sup>. Hence by considering the above facts, the fruits of *Grewia subinaequalis* were selected for the screening of *invitro* antiarthritic activity. Most of the medicinal value of *Grewia* species is due to the presence of various secondary metabolites like saponins, coumarins and anthraquinones<sup>6</sup>. The fruits and roots are well known remedies for the treatment of osteoporosis, tissue and wound healing<sup>6</sup>. They have free radical scavenging activities which may be responsible for the therapeutic action against the tissue damage<sup>7</sup>. The fruit has a high content of antioxidants like Vitamin C, total phenolics, flavonoids, tannins and anthocyanins<sup>8</sup>. The antioxidant activity of *Grewia subinaequalis* can be explained on the basis of total phenolic contents, flavonoids and anthocyanins<sup>9</sup>. It is also established that antioxidant activity of lots of fruits are based on their flavonoid content<sup>10</sup>. The plant is also reported as antimicrobial<sup>11</sup>, anticancer<sup>12</sup>, Radio protective agent<sup>13</sup>, Hepatoprotectant<sup>14</sup> and Antiviral activity<sup>15</sup>. Since the plant is also reported with anti-inflammatory activity<sup>16</sup> an attempt has been carried out to screen the aqueous and alcoholic (ethanolic) extract of the fruits for its *invitro* antiarthritic activity.

## 2. MATERIAL AND METHODS

### Plant Material

The matured fruits of *Grewia subinaequalis* were collected from the forest areas of Thirunelli, Wayanad district of Kerala state. It was then shade dried and powdered after confirming the botanical identity. A voucher specimen (GSfr.14) has been deposited at the Department of Pharmacognosy and Phytochemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

### Preparation of The Fruit Extracts

The powdered fruits were subjected to extraction by using water as well as ethanol. The aqueous extract as well as

ethanolic extracts were screened for the presence of various phytoconstituents<sup>17</sup>.



**Fig 1: Plant showing the matured fruit of *Grewia subinaequalis***

## EVALUATION OF ANTI-ARTHRITIC ACTIVITY

### Denaturation of Proteins By Egg Albumin

The 5ml of reaction mixture consists of 0.2ml of egg albumin obtained from the fresh hen's egg, 2.8ml of phosphate buffered saline of P<sup>H</sup> 6.04 and 2ml of varying concentrations of aqueous and ethanolic extracts of the fruits of *Grewia subinaequalis* so that the final concentration become 100, 200, 400, 800 and 1000 µg/ml. Similar volume of distilled water served as control. Then the mixtures were incubated at 37±2 °C in a BOD incubator for 15 minutes and then heated at 70°C for 15 minutes. After cooling, their absorbance was measured at 660nm. Acetyl salicylic acid was used as reference standard<sup>18</sup>. The percentage of inhibition of protein denaturation was calculated by using the following formula;

Percentageinhibition

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

### Denaturation Of Proteins By Bovine Albumin

The reaction mixture was consisting of aqueous and ethanolic extract of the fruits of *Grewia subinaequalis* at different concentrations and 1% of aqueous solution of bovine albumin. The samples were incubated at 37°C for 20 minutes and then heated at 57°C for 20 minutes after cooling the samples. Absorbance of turbidity was measured at 660nm<sup>19</sup>. The percentage of inhibition of protein denaturation was calculated by using the following formula;

Percentageinhibition

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

### Evaluation of Anti-Microbial Activity

The matured fruit after drying were crushed and powdered. Extraction was carried out by soxhlet method using ethanol as solvent. The micro organisms were collected from the department of Microbiology, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

Susceptibility testing of the microorganisms were performed by using *Escherichia coli* and *Lactobacillus acidophilus*. Antimicrobial susceptibility testing was done using agar well diffusion method<sup>20</sup> to detect the presence of antimicrobial activity of the ethanolic extract of the fruit of the plant. Nutrient agar slants after solidification was inoculated with the test micro organism, by spreading the bacterial inoculums under aseptic conditions. Wells of 5mm diameter were punched in the agar medium with sterile cork borer and filled with the ethanolic extract of the fruit of the plant. The test bacterial strains were inoculated into Mueller Hinton agar medium. The different concentrations of the ethanolic fruit extract were filled inside the wells and the plate was transferred to a refrigerator at 15<sup>0</sup>C for about 10 to 20minutes for diffusion there after the plate was incubated for 24 hours at 37<sup>0</sup>C. The anti bacterial activity of the ethanolic extract of the fruit was expressed in terms of zone of inhibition in mille meter (mm). The minimum inhibitory concentration was determined at the lowest concentration of the fruit extract inhibiting the growth of the organism<sup>21</sup>.

**3. RESULTS**

In the present study, the aqueous and alcoholic (ethanolic) extracts of the fruits of *Grewia subinaequalis* and acetyl salicylic acid (reference drug) were investigated for antiarthritic activity by protein denaturation method. The maximum antiarthritic activity was observed in in the concentration 1000 µg/ml, while the minimum activity was observed in the concentration 100 µg/ml. The *invitro* antiarthritic activity of the fruits of *Grewia subinaequalis* by egg albumin method is shown in table 1, where, the percentage of arthritic protection was found to be 90.6 (aqueous), 93.1(alcoholic/ethanolic) in 1000ml concentration and 88.7 for aspirin. Almost similar results were obtained in the protein denaturation method using bovine albumin method and are tabulated in table 2. The inhibition of protein denaturation of bovine albumin was found to be 97.4 (aqueous), 89.9 (alcoholic/ethanolic) and that of acetyl salicylic acid was found to be 86.8. From the findings it is very clear that both the aqueous and alcoholic (ethanolic) extracts of the fruits of *Grewia subinaequalis* exhibited a dose dependent response and also it can be assumed that the alcoholic (ethanolic) fruit extract possess prominent and significant antiarthritic activity when compared with the aqueous extracts of the fruits of the same plant. The effects of the antiarthritic activity of both aqueous and ethanolic extracts of the fruits were comparable with the acetyl salicylic acid which was used as a reference standard during the evaluation.

The antimicrobial evaluation of the ethanolic extract of the fruits reveals the capacity of the fruits to inhibit or decrease the growth of the pathogenic microorganisms. The presence of antimicrobial constituents in plants is well established as they have provided a source of inspiration for novel drug compound as plant derived medicines. The zone of

inhibition at its maximum was recorded in 2000 µg/ml and that of minimum was 250 µg/ml for both the bacteria screened for the evaluation. The zone of inhibition obtained was depending up on the various concentration of the ethanolic extract of the fruit. The study clearly indicates the presence of antibacterial principles present in the ethanolic extract of the fruit of the plant.

**Anti-Arthritic Activity**

**Table 1: Percentage inhibition of protein denaturation of the fruits of *Grewia subinaequalis* using egg albumin**

Treatment Regimen	Concentration (µg/ml)	Percentage of Inhibition (%)
Fruit extract of (Aqueous) <i>Grewia subinaequalis</i>	100	16.4
	200	31.2
	400	50.3
	600	67.6
	800	71.4
	1000	90.6
Fruit extract of (Alcoholic/Ethanolic) <i>Grewia subinaequalis</i>	100	19.7
	200	28.6
	400	45.9
	600	57.6
	800	62.1
	1000	93.1
Acetyl salicylic acid/Aspirin (Reference standard)	50	88.7

**Table 2: Percentage inhibition of protein denaturation of the fruits of *Grewia subinaequalis* using bovine albumin**

Treatment Regimen	Concentration (µg/ml)	Percentage of Inhibition (%)
Fruit extract of (Aqueous) <i>Grewia subinaequalis</i>	100	18.3
	200	29.7
	400	44.4
	600	69.2
	800	81.5
	1000	97.4
Fruit extract of (Alcoholic/Ethanolic) <i>Grewia subinaequalis</i>	100	16.8
	200	27.4
	400	51.2
	600	63.4
	800	70.6
	1000	89.9
Acetyl salicylic acid/Aspirin (Reference standard)	50	86.8

**Anti- Microbial Activity**

**Table 3: Antimirbial activity of the ethanolic extract of the fruit of *Grewia subinaequalis***

Micro organisms used	Concentration of the ethanolic extract of the fruits(µg/ml)	Zone of Inhibition (mm)
	250	21
	500	25

<i>Escherichia coli</i>	1000	29
	1500	31
	2000	34
<i>Lactobacillus acidophilus.</i>	250	19
	500	22
	1000	27
	1500	31
	2000	33

#### 4. DISCUSSION

The antiarthritic activity exhibited by the fruit extracts could be due to the presence of flavonoids, phenolic compounds and vitamin C. Denaturation of tissue protein is one of the well documented cause of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to *invivo* protein denaturation<sup>22,23</sup>. Hence the drugs which can prevent the denaturation of protein would be worth for establishing the antiarthritic and anti-inflammatory drug development. The mechanisms of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding<sup>24</sup>. Most of the medicinal value of *Grewia* species is due to the presence of various secondary metabolites like saponins, Phenolic compounds, flavonoids coumarins and anthraquinones<sup>6</sup>. The antimicrobial effects produced by the fruits could be due to the presence of flavonoids and phenolic compounds.

#### 5. CONCLUSION

From the results of the present study, it can be stated that the fruit extracts of *Grewia subinaequalis* is capable of controlling the production of auto antigens due to *invivo* denaturation of proteins in rheumatic diseases. Further the isolation of lead molecules responsible for the antiarthritic activity has to be carried out which may be beneficial for the development of new natural antiarthritic agents with less side effects compared to synthetic molecules. The alcoholic (ethanolic) extract of the fruit also possess potent antimicrobial action. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

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