

Phytochemical screening, anti-pyretic and antidiarrhoeal activities of the n-hexane and aqueous extracts of the leaves of *Aegle marmelos*

Hameed M. A.^{1,3}, **Faheem Amir**^{*2}, Koay Yen Chin², Shaheedha Fathima³

¹ Faculty of Pharmacy, Universiti Teknologi MARA, Selangor 42300, Malaysia

² School of Chemical Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia

³ Sultan-ul-uloom College of Pharmacy, J.N.T.U, Hyderabad, A.P., India

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Abstract

Objective: The n-hexane and aqueous extracts of the leaves of *Aegle marmelos* have been examined anti-pyretic and antidiarrhoeal activities, and phytochemical screening.

Materials and methods: Yeast-induced hyperthermia test was used for antipyretic activity. For both the extracts, rectal temperatures were measured at fixed intervals. Castor oil-induced diarrhoea test was used for antidiarrhoeal activity. For both the extracts, the droppings were collected at fixed intervals. The phytochemical screening was done according to the methods described in the literature.

Result: The results indicated good anti-pyretic activity for the aqueous extract and good antidiarrhoeal activity for the n-hexane extract of *Aegle marmelos*.

Conclusion: The results of the study supported the traditional use of plant as a crude anti-pyretic and antidiarrhoeal drug.

Key words

Aegle marmelos, anti-pyretic, antidiarrhoeal, phytochemical screening

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Corresponding Author

Faheem Amir

School of Chemical Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia

Email: faheem.a42@gmail.com

Introduction

Aegle marmelos belongs to the family Rutaceae. It is a medium sized tree, widely distributed in Asia and Africa [1-2]. The plant is widely used in the Ayurvedic system of medicine. Traditionally the plant has been used for the treatment of various ailments such as pain, fever, inflammation, respiratory

disorders, cardiac disorders, dysentery and diarrhoea; it is also used as a digestive and febrifuge [3-6]. The leaves of the plant are reported to possess anti-cancer, anti-hyperglycemic, anti-inflammatory, anti-pyretic, analgesic, anti-diabetic, anti-spermetogenic, anti-bacterial, antidiarrhoeal and chemopreventive activities [2, 7-9].

The leaves, amongst other properties indicated predominant anti-pyretic and antidiarrhoeal properties. These activities have been reported previously, however this study was conducted since these properties have not been reported for the n-hexane and aqueous extracts of the leaves [9]. Furthermore, phytochemical screening was conducted to identify the classes of compounds responsible for these activities.

Materials and Methods

Plant materials

The leaves of *Aegle marmelos* were collected from Hyderabad, Andhra Pradesh, India. A voucher specimen has been deposited in the herbarium of the same University.

Animals

Wistar albino rats of either sex weighing (150-180 g) were kept at 25 ± 2°C, under standard husbandry conditions and provided standard food and water.

Preparation of extracts

The shed dried leaves (500g) were extracted successively by soaking in n-hexane for 18 hours at room temperature, followed by water for 24 hours, to yield n-hexane extract (3.5 g) and aqueous extract (8.5 g). The extracts were evaporated under reduced pressure using a rotary evaporator; all the extracts were freshly prepared before use.

Phytochemical screening methods

For alkaloid analysis the extracts were treated with 1% HCl, boiled and filtered. Dragendorff's, Hager's, Mayer's and Wagner's reagents were used to indicate the presence of alkaloids [10-11], Libermann Burchard and Salkowski tests for the presence of triterpenoids and steroids, respectively, foam test for the presence of saponins, Molisch test for the presence of carbohydrates, Brontrager test for the presence of anthroquinone glycoside, Baljet test, Keller-Killiani and Legal for the

presence of cardiac glycosides, and alkaline test for the presence of coumarins. The presence of flavonoids was determined by treating the methanol extract with magnesium turnings, and adding HCl drop-wise, formation of magenta colour indicating the presence of flavonoids [10-13]. For the presence of tannins was indicated by Gelatin-salt Block test [13-14]. Phenolics were determined by adding 1% aqueous ferric chloride to the alcoholic or aqueous extracts, appearance of intense green, purple, blue or black colours indicating the presence of phenolics [10]. Cardiac glycosides were identified by treating the alcoholic extract with 2 ml glacial acetic acid containing 1 drop of 5% FeCl₃ solution, this solution being applied to the surface of 1 ml conc. H₂SO₄. Formation of a reddish brown ring between the two layers indicated the presence of cardiac glycosides [10, 15]. Benedict and Fehling test and were used for the presence of sugars, Enrich test for the presence of furanoids, and saponification and spot test for the presence of fixed oils and fats. For the presence of catechins a portion of match stick was dipped into the extract and then into concentrated HCl formation of pink to red on the wooden portion of the stick indicated the presence of catechins. Biuret, Millons and Ninhydrin test indicated the presence of proteins. The volatile oil of the extracts was determined by the hydrodistillation of the extracts. The extracts were treated with NaOH formation of blue-green or red colour indicated the presence of quinones. The aqueous or alcoholic extracts were treated with NaOH solution; formation of blue-violet colour indicated the presence of anthocyanins [10-11, 16-18].

Anti-pyretic test (yeast-induced hyperthermia in rats)

Wistar albino rats of either sex weighing 150–180 g were divided into four groups of five animals each. Rats were given subcutaneous injection of 20 ml/kg body weight of 20% aqueous suspension of sterilized brewer's yeast powder [19]. After 18 hours, animals showing satisfactory increase of rectal temperature (> 0.5 °C) were selected. Control group received 10% DMSO (oral); standard group received 150 mg/kg of paracetamol (oral) and treated groups received 300 mg/kg of extracts (oral). Rectal temperature was determined by thermal-probe at 30 min, 1, 2, 3, 4 hours, after administration.

Antidiarrhoeal test (castor oil-induced diarrhoea in rats)

The rats were fasted for 18 hours, then group I (Received 10% DMSO) served as control, Group II received the standard atropine 3 mg/kg, Group III and IV received the test extracts (each 300 mg/Kg). One hour after administration all animals received 1 ml of castor oil orally. The presences of characteristic diarrhoeal droppings were noted in the transparent plastic dishes placed beneath the individual rat cages. The numbers of droppings were calculated at one hour intervals for four hours after castor oil administration. The effects of the test drug, standard antidiarrhoeal agent atropine were calculated based on the frequency of defecation by comparing with control rats [20-21].

Statistical analysis

The values were expressed as mean±SEM. Statistical analysis and comparison between the groups was performed by one way analysis of variance (ANOVA) using SPSS version 10.0, followed by LSD (Least Significance Difference) test. Difference between unexposed and exposed (with or without treatment)

with a *p*-value < 0.01 was considered significant.

Results and Discussion

Phytochemical screening

The preliminary phytochemical screening showed the presence of cardiac glycosides, steroids, triterpenoids and pseudo tannins in the n-hexane extract, and alkaloids, anthroquinone glycoside, catechins, fixed oils and fats, furanoids, proteins, phenolics and saponins in the aqueous extract (Table 1).

Anti-pyretic activity

The inhibitory effect of a drug on prostaglandin-biosynthesis are known as anti-pyretic activity [19]. The Brewer's yeast induced pyrexia in rats was found to reduce significantly with the aqueous extract. However, no significant reduction in body temperature was observed for the n-hexane extract (Table 2, Figure 1).

Antidiarrhoeal activity

Recinoleic acid the active constituent of castor oil is known to cause diarrhoea [22], several mechanisms of action have been proposed however the correct mechanism has not been reported yet [20]. The defecation count was found to be significantly decreased in n-hexane extract when compared with control; this antidiarrhoeal effect of n-hexane extract was similar to that of the standard. But there was no significant reduction of defecation count in aqueous extract (Table 3, Figure 2).

Conclusion

The leaf extracts of *A. marmelos* showed anti-pyretic and antidiarrhoeal activities. The results of this study support the crude anti-pyretic and antidiarrhoeal nature of the plant. For future studies it would be valuable to isolate the biologically active constituents responsible for these activities.

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Table 1 Phytochemical screening of the n-hexane and aqueous extracts of *Aegle marmelos*

Class of compounds	n-Hexane extract	Aqueous extract
Alkaloids	-	+
Anthocyanins	-	-
Anthroquinone glycoside	-	+
Carbohydrates	-	-
Cardiac glycosides	+	-
Catechins	-	+
Condensed tannins	-	-
Coumarins	-	-
Fixed oils and fats	-	-
Flavonoids	-	+
Furanoids	-	+
Phenolics	-	+
Proteins	-	+
Pseudo tannins	+	-
Quinones	-	-
Saponins	-	+
Steroids	+	-
Sugars	-	-
Triterpenoids	+	-
Volatile oils	-	-

+ (present); - (absent)

Table 2 Anti-pyretic activity of the n-hexane and aqueous extracts of *Aegle marmelos*

	0 hrs	30 min	1 hr	2 hrs	3 hrs	4 hrs
Control	38.92±0.07	38.96±0.05	39.06±0.06	39.14±0.04	39.18±0.03	39.2±0.03
Standard	39.98±0.58	38.68±0.08	38.34±0.10	37.8±0.08	37.42±0.04	37.02±0.07
Test 1 (Aq. extract)	38.92±0.06	38.64±0.04	38.24±0.08	37.84±0.06	37.68±0.20	36.96±0.02
Test 2 (Hex. extract)	38.92±0.05	38.70±0.08	38.62±0.07	38.46±0.09	38.32±0.09	38.28±0.08

Control vs Test2 = P < 0.01; Control vs Standard = P < 0.01; Control vs Test 1 = P < 0.01

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