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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON WHOLE PLANT OF *ASYSTASIA GANGETICA*

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ABSTRACT

Asystasia gangetica (Linn) T.Anderson belonging to the family Acanthaceae is an ornamental plant and used as a vegetable in times of food scarcity. It is having many medicinal properties and is used in folklore medicine for various ailments like asthma, rheumatism, swellings etc. The whole plant of Asystasia gangetica was used for the study. The solvent methanol was used for the extraction as it was reported to extract the maximum phytoconstituents. The preliminary phytochemical analysis indicated the presence of compounds like Flavonoids, Alkaloids, Glycosides, Coumarins, Tannins, Steroids, Terpenoids and Saponins. The nutritive value of the methanolic extract was evaluated by estimating the Carbohydrate, Protein and Lipid content. The results justified its use as a vegetable in times of food scarcity. The secondary metabolites content like Phenols, Tannin and Flavonol content was also estimated.

The Methanolic Extract of the whole plant was subjected to fractionation by Column chromatography with solvents of graded polarity. A Flavonoid Mixture Fraction was obtained in the Ethyl acetate: Methanol (50: 50) fraction. This mixture was characterised by chemical tests, Thin Layer Chromatography and High Performance Liquid Chromatography. The HPLC characterisation reported the presence of 4 known Flavonoids and 2 unknown compounds. The four Flavonoids were Luteolin, Quercetin, Kaempferol and Isorhamnetin. The in- vitro pharmacological activities were performed for the methanolic extract of the whole plant of Asystasia gangetica.

The methanolic extract was selected for the pharmacological activities because it showed the maximum anti-inflammatory effect as reported in literature. Protein Denaturation produces auto antigens which may be a cause for the development of Rheumatoid arthritis. The study of Anti arthritic activity of the methanolic extract showed a dose dependent inhibition of protein Denaturation and was comparable to that of the standard. The methanolic extract also exhibited a dose dependent inhibition of ADP induced platelet aggregation. The methanolic extract was also evaluated for its Anthelmintic activity. It showed a dose dependent decrease in time taken for paralysis and death of worms. The methanolic extract and Flavonoid Mixture fraction were subjected to tests on blood viscosity. The Flavonoid mixture fraction exhibited a better decrement in blood viscosity than the methanolic extract.

KEY WORDS: *Asystasia gangetica,* Anti-Oxidant, Anti-Arthritic, *In-vitro,* Anthelmintic

INTRODUCTION

Plants play a pivotal role in health care. According to World Health Organisation (WHO), 80% of the world's population relies on traditional medicine, particularly plant drugs for primary health care. The practise of traditional medicine is not new in India since it is the birth place of many traditional practices like Ayurveda, Siddha and Unani. India is particularly well endowed with over 6000 medicinal plants and well recorded practical knowledge of traditional medicine. The scientific mind will not be satisfied by mere claims no matter from whatever source they originate, unless corroborated by experimental and clinical evidences. As it is evident that plants are treasure house for many potent medicines, it is important to scientifically evaluate the traditional practices as well as upgrade the existing knowledge and make it available to the general public.

The very important plant molecules like Digitoxin, Morphine, Vincristine, Quinine etc have been used as prototypes for the discovery of newer synthetic molecules. The frequency of life threatening diseases and ailments has increased worldwide and it is becoming an important cause of morbidity and mortality in developing countries. Majority of the diseases like Atheroscelorosis, Arthritis, Diabetes, and Cancer occur due to free radical generation. Plants contain naturally occurring anti-oxidants like Flavonoids, Tannins etc which can be utilised for scavenging free radicals. Many of the plant Alkaloids affect the nervous system and hence have been used as Anaesthetics, Psycho stimulants, Motor end Depressants etc.

Asystasia gangetica is an ornamental plant and has been used as a source of nutrition in times of food scarcity. It is also used traditionally for many ailments and diseases. Already the traditional use of the leaves as anti-asthmatic (Akah PA, 2003) was scientifically proven. But no work on its anti-arthritic, Anthelmintic and anti-

platelet properties was performed. It was in this context that the multiple pharmacological activities of the whole plant of *Asystasia gangetica* were investigated in the course of this study. Also an attempt was made to isolate the possible bioactive molecule responsible for the pharmacological actions. *Asystasia gangetica* (Linn.) T.Anderson is commonly known as Chinese violet. 'Asystasia' means inconsistency and it relates to the fact that the corolla is slightly regular which is an unusual characteristic in the Acanthaceae family. The word 'gangetica' is derived from Ganges River where it is presumed to occur. It is locally used as a potherb and leafy vegetable in times of food scarcity. It is also promoted as cover plant in orchards as it checks soil erosion and prevents growth of noxious weeds. It also attracts bees to orchards. It has high nutritive value and hence used as forage for cattle, goats and sheep in south East Asia. It is also used as an ornamental plant.

MATERIALS AND METHODS

a. Plant material: The whole plant was collected from Tirumala hills, Andhra Pradesh, in the month of December 2009. The plant material was authenticated by National Institute of Herbal Science (PARC/2010/542), West Tambaram, Chennai.

b.Preparation of the extract: The freshly collected whole plant was dried in shade and coarsely powdered with a blender. 200 grams of the powder was subjected to continuous hot percolation using Soxhlet's apparatus with the solvent methanol for 48 hrs. The solvent was recovered by distillation in Rotary Vacuum Evaporator at 80^{o}C. The residue was stored in a desiccator and used in further studies.

Fig 1: Aerial parts of *Asystasia gangetica*

Fig *2: Asystasia gangetica* habit

c. Phytochemical Evaluation: Compounds like Flavonoids, Alkaloids, Tannins, and Glycosides etc. are responsible for many pharmacological activities of a plant. Phytochemical evaluation gives the chemical nature of the bioactive molecules responsible for pharmacological activity in a plant.

d. Pharmacological studies: The following Pharmacological studies has been carried out on the whole plant of *Asystasia gangetic such as* determination of Anti-arthritic activity by inhibition of protein denaturation (Mizushima et al., 1966), *In - vitro* determination of *anti-platelet* activity by inhibition of platelet aggregation induced by ADP, *In - vitro anthelmintic* activity (Fabiyi, 1986) and Effect of plant extract on blood viscosity.

RESULT AND DISCUSSION

In- Vitro Pharmacological Activities

1. Anti-arthritic activity by inhibition of protein denaturation method: The percentage of inhibition of protein Denaturation of the methanolic extract and the standard Diclofenac sodium are tabulated in **Table.1** & represented in **fig 3.**

Table.1 Percent inhibition of Protein Denaturation by Methanolic Extract & Diclofenac sodium

Concentration µg/ml	Percentage Inhibition of Methanolic extract	Percent Inhibition of Diclofenac sodium
10	17.29	-
50	23.61	-
100	33.34	-
200	42.70	84.47
400	58.11	-
800	65.87	-
1000	78.94	-

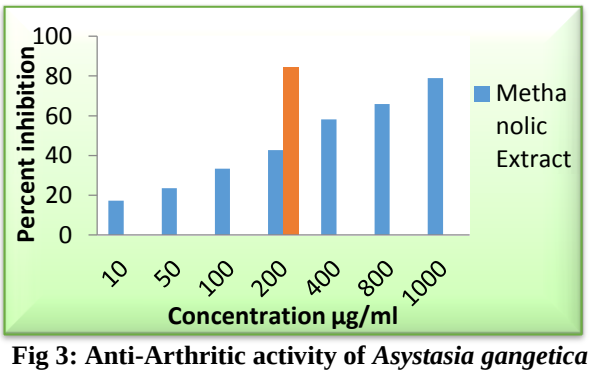


Fig 3: Anti-Arthritic activity of *Asystasia gangetica*

The methanolic extract exhibited a dose dependent inhibition of Protein Denaturation for Anti Arthritic activity. The maximum percent inhibition (78.94%) was exhibited by 1000 µg/ml of the methanolic extract.

2. Anti-platelet activity by inhibition of ADP induced platelet aggregation: The percentage transmittance of the methanolic extract and the standard drug aspirin in different time intervals are tabulated in **Table.2** & represented in **fig 4**

Table.2 Percent transmittance of methanolic Extract & Aspirin for Anti-Platelet Activity

Concentration (µg/ml)	Percentage transmittance					
	0min	1min	2min	3min	4min	5min
100	2.311	2.433	2.453	2.546	2.743	2.764
200	3.764	3.001	3.437	3.813	3.760	3.941
400	4.111	4.024	4.512	4.589	4.708	4.800
500	5.452	5.675	5.876	5.783	5.863	5.944
ASPIRIN	6.769	6.654	6.540	6.502	6.471	6.411

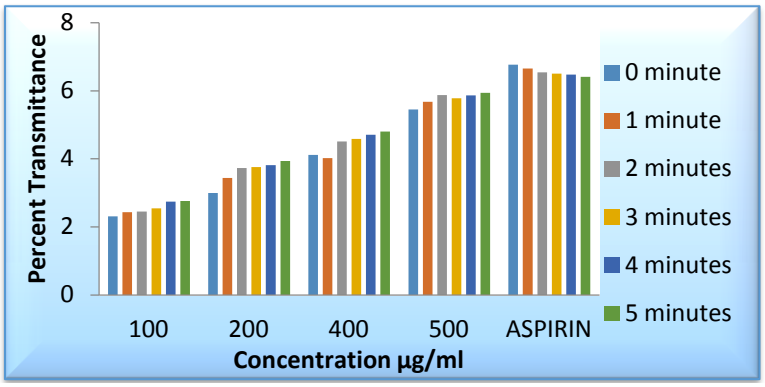


Fig 4: Percent transmittance of Methanolic Extract & Aspirin for Anti-Platelet Activity

The methanolic extract showed a dose dependent inhibition of aggregation for Anti Platelet activity and the maximum inhibition was exhibited by 500 µg/ml of Methanolic extract.

3. *In- Vitro* Anthelmintic Activity

The average values of the time taken for paralysis and death of *Pheretima posthuma* are tabulated in **Table.3** and represented in fig 5

Table.3 Time taken for Paralysis & Death in Albendazole and Methanolic Extract treated groups

Group	Solution	Concentration (mg⁄ml)	Time taken for paralysis (min)	Time taken for death (min)
I	Control Solution	-	-	-
I	Albendazole (Standard)	10	38±0.3	56±0.3
I	Test Extract	10	45±0.2	54±0.5
		50	37±0.6	43±0.3
		100	25±0.4	30±0.8
		200	19±0.9	24±0.4

Values are Mean± SEM of 6 observations.

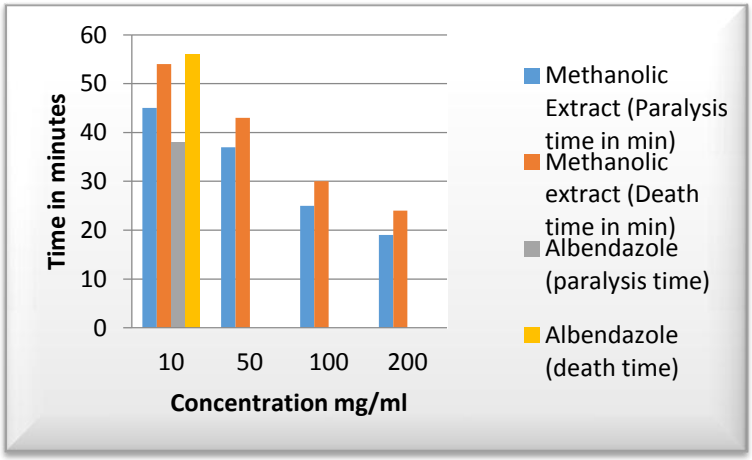
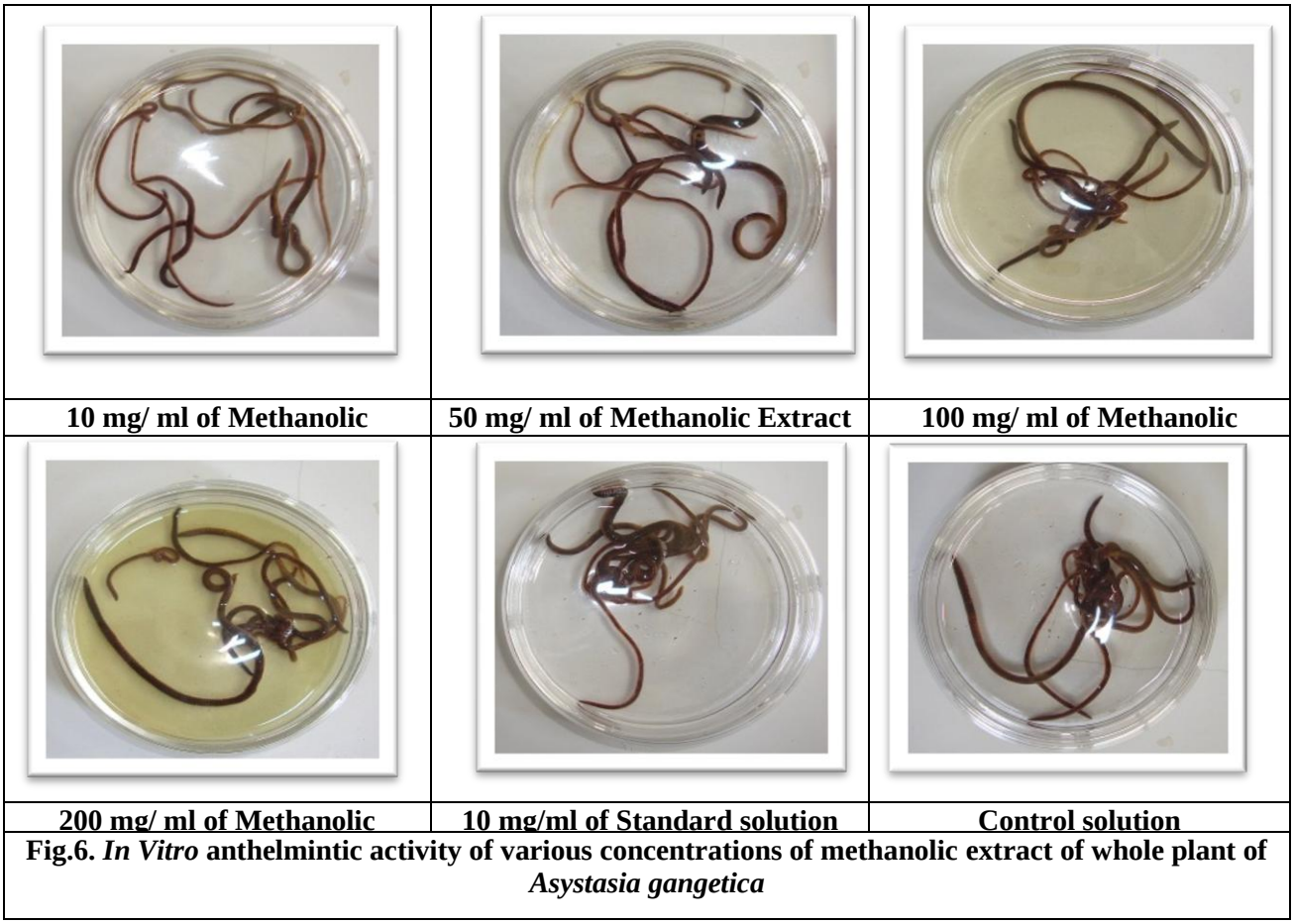


Fig 5: Time taken for Paralysis & Death in Albendazole and Methanolic Extract treated groups

The Methanolic extract showed a dose dependent decrease in time taken for paralysis and death of *Pheretima posthuma*. The maximum dose of 500 mg/ml of methanolic extract showed the least time taken for paralysis and death of the earth worms.



10 mg/ ml of Methanolic	**50 mg/ ml of Methanolic Extract**	**100 mg/ ml of Methanolic**
200 mg/ ml of Methanolic	**10 mg/ml of Standard solution**	**Control solution**

Fig.6. *In Vitro* anthelmintic activity of various concentrations of methanolic extract of whole plant of *Asystasia gangetica*

4) Effect of methanolic extract and flavanoid mixture fraction on blood viscosity

The viscosity of Methanolic extract treated blood is tabulated in **Table.4** and represented in **fig 7.**

Table.4 Effect of Methanolic Extract on Blood Viscosity in time span of 90 minutes

Concentration (µg/ml)	0 minute	30 Minutes	60 minutes	90 minutes	Relative blood viscosity
100	2.2	2.2	2.1	2.1	2.2
200	2.1	2	2	1.9	
300	1.9	1.9	1.9	1.8	
400	1.8	1.7	1.7	1.6	
500	1.6	1.6	1.5	1.5	

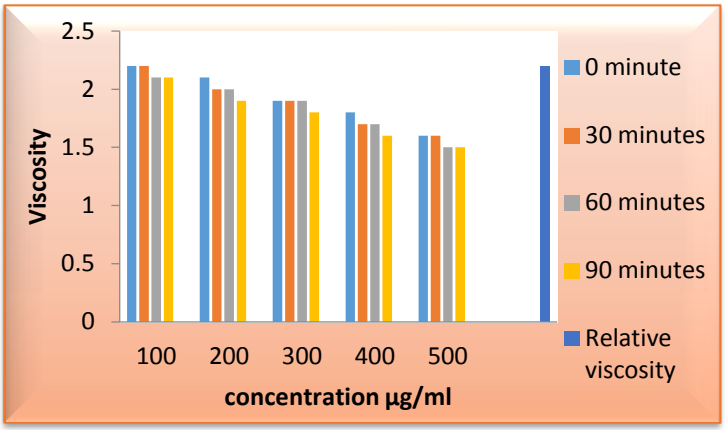


Fig: 7 Effect of Methanolic extract on Blood viscosity

The effect of various concentrations of Flavanoid fraction on blood viscosity at various time intervals is tabulated in **Table.5** and represented in **fig 8.**

Table.5 Effect of Flavonoid Mixture Fraction on Blood viscosity in time span of 90 minutes

Concentration (µg/ml)	0minute	30minutes	60minutes	90minutes	Relative blood viscosity
100	2.2	2.1	1.9	1.9	2.2
200	1.9	1.8	1.8	1.7	
300	1.7	1.7	1.6	1.6	
400	1.6	1.5	1.5	1.5	
500	1.5	1.5	1.4	1.4	

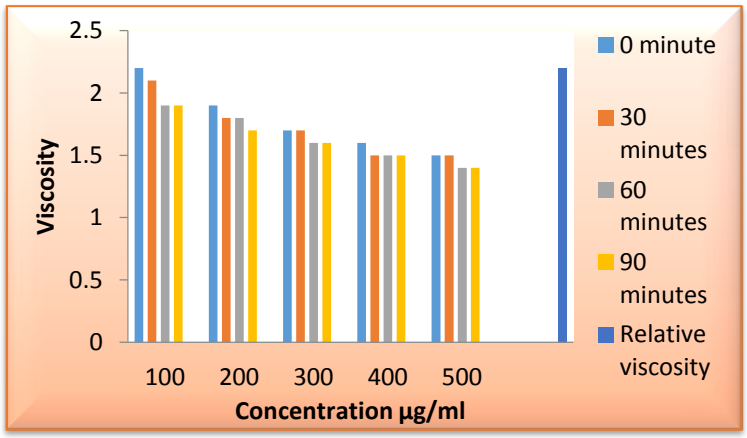


Fig 8: Effect of Flavonoid Mixture Fraction on Blood viscosity

The methanolic extract showed a dose dependent decrease in Blood viscosity in a span of 90 minutes. The Flavanoid Mixture fraction showed better decrease in viscosity than methanolic extract.

CONCLUSION

The study justified the use of the plant as a food source by quantifying the nutritive value. The study also justified the use of plant as Anthelmintic and anti-Arthritic in folklore medicine. The methanolic extract also exhibited inhibition of platelet aggregation and decrease in blood viscosity. Also this is the first report of the flavonoids Luteolin, Quercetin, Kaempferol and Isorhamnetin being isolated from the Methanolic Extract of the whole plant of Asystasia gangetica. The study also reports that the decrease in blood viscosity by the methanolic extract was due to the Flavonoid mixture, which was isolated by column chromatography.

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