IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2018 Vol.:12, Issue:1

Phytochemical Screening and Anthelmintic Activity Assay of Drakshadi Kashayam

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Revathy. C*, N. L Gowrishankar, Adisha. S, Aiswarya. N. K, Asna. M, Krishnapriya P.G, Mohammed Junaid. K

Prime College of Pharmacy, Prime Nagar, Erattayal, Kerala 678551

Submission: 27 March 2018
Accepted: 03 April 2018
Published: 30 April 2018

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Drakshadi kashayam; anthelmintic activity, phytochemical constituents; *Pheretima posthuma*.

ABSTRACT

The present investigation was aimed at instigating the anthelmintic activity of Drakshadi kashayam, an Ayurvedic formulation, using Indian earthworms *Pheretima posthuma* as test worm. The formulation was subjected to preliminary phytochemical screening in order to find out the major phytoconstituents present in the preparation. Various concentrations of the formulation (50 mg/ml and 100 mg/ml) were tested which involved determination of paralysis time and death time of the worm. Albendazole (100mg/ml) was used as the reference standard. The result of the present study indicates that the formulation showed the presence of various phytoconstituents such as alkaloids, tannins, flavonoids, steroids, glycosides etc and it exhibited dose dependent & significant anthelmintic activity. The higher concentration of formulation was found to possess better anthelmintic activity as compared to the formulation at a lower concentration. The anthelmintic activity of the formulation was comparable with that of the activity possessed by the standard drug Albendazole.

INTRODUCTION

Ayurveda is a system of remedy with chronological ancestry in the Indian subcontinent. Globalized and rationalized practices gained from Ayurveda traditions are a type of alternative or complementary medicine. In countries beyond India, Ayurvedic healing and practices have been integrated in general wellness applications and in some cases in medical use.[1] Ayurveda has historically divided corporal substances into five classical elements (*[maha]panchabhuta*, viz. Fire, water, air, earth and ether).[2]

Indian medication, plant formulations and collective extracts of plants are selected rather than individual ones. It is well-known that Ayurvedic herbals are prepared in a number of dosage forms, in which mostly all of them are PHF. Synergism, polyherbalism awards some advantages that are not present in single herbal formulation. PHFs take to improved handiness for patients by purging the need of consuming more than one different sole herbal formulation at a time, which circuitously leads to enhanced compliance and therapeutic effect. All these benefits have resulted in the popularity of PHF in the market when put side by sided to single herbal formulation. In the preparation of polyherbal formulations, it is vital to note that herbs are sometimes judged to be incompatible (viruddha) and thus should never be taken simultaneously. Such incompatibility may be because of energetic incompatibility, functional incompatibility or quantitative incompatibility. Safety, effectiveness, ubiquity, cheap and better acceptance, have made PHF the perfect treatment of choice, hence superior compliance by the patients and tremendous therapeutic outcome is ensured.[3]

Anthelmintics or antihelminthics are a group of antiparasitic drug that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them, without causing significant damage to the host. They may also be called vermifuges (those that stun) or vermicides (those that kill). Anthelmintics are used to treat people who are infected by helminths, a condition called helminthiasis.[4]

MATERIALS AND METHODS

Drakshadi kashayam (Batch number-516716) was procured from a standard ayurvedic shop from Kerala.

1. Preparation of Formulation

The formulation was diluted to the suitable concentrations with the help of distilled water.

2. Preliminary Phytochemical Screening

The formulation was subjected to standard phytochemical screening tests for various constituents.

2.1. Test for alkaloids

Small amount of formulation was mixed with few ml of dilute hydrochloric acid. Shaken well and filtered.

2.1.1. Dragendorff's test

A few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to 2-3ml of filtrate. Orange red precipitate indicates the presence of alkaloids.

2.1.2. Mayer's test

A few drops of Mayer's reagent (potassium mercuric iodide solution) were added to 2-3ml of filtrate. Cream (dull white) precipitate indicates the presence of alkaloids.

2.1.3. Wagner's test

A few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to 2-3ml of filtrate. Reddish brown precipitate indicates the presence of alkaloids.

2.1.4. Hager's test

A few drops of Hager's reagent (Picric acid) were added to 2-3ml of filtrate. Yellow precipitate indicates the presence of alkaloids.

2.2. Test for carbohydrates

2.2.1. Molisch's test

Few drops of Molisch's reagent were added to 2-3ml of filtrate, followed by addition of concentrated sulphuric acid along the sides of the test tube. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates.

2.2.2. Fehling's test

1ml Fehling's-A (copper sulphate in distilled water) was added to 1ml of Fehling's-B (potassium tartarate and sodium hydroxide in distilled water) solution, boiled for one minute. To this added 1ml of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

2.2.3. Benedict's test

Few ml of filtrate was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex) and heated in boiling water bath for 5min. Formation of reddish brown precipitate infers the presence of reducing sugars.

2.3. Test for triterpenoid

2.3.1. Libermann-Burchard test

A small quantity of formulation was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green colour, infers the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

2.3.2. Salkowski test

A small quantity of the formulation was treated with chloroform and few drops of concentrated sulphuric acid and allowed to stand for few minutes. Yellow colour at the lower layer indicates the presence of triterpenoids.

2.4. Test for steroids and sterols

2.4.1. Liebermann- Burchard reaction

2ml of formulation was mixed with chloroform. To that mixture added 1-2ml of acetic anhydride and 2 drops of concentrated sulphuric acid along the sides of the test tube. The solution becomes red, then blue and finally bluish green colour.

2.4.2. Salkowski reaction

2ml of formulation was mixed with 2ml chloroform and 2ml concentrated sulphuric acid. Shaken well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

2.5. Test for saponins

2.5.1. Foam test

1ml of test sample was diluted with 20ml of distilled water and shaken it in a graduated cylinder for 3minutes. Foam of 1cm after 10min indicates the presence of saponins.

2.5.2. Froth test

5ml of test sample was added to sodium bicarbonate solution. After vigorous shaking the mixture kept it for 3minutes. A honeycomb like froth formation indicates the presence of saponins.

2.6. Test for glycosides

2.6.1. Legal's test

1ml of pyridine and 1ml of sodium nitroprusside was added to 1ml of extract. Pink to red colour indicates the presence of glycosides.

2.6.2. Keller-Killiani test

Glacial acetic acid was added to 2ml extract, followed by the addition of trace quantity of ferric chloride and 2 to 3drops of concentrated sulphuric acid. Reddish brown colour appears at the junction of two liquid indicates the presence of cardiac glycosides.

2.6.3. Baljet test

2ml of extract was added to sodium picrate solution. Yellow to orange colour formation indicates the presence of glycosides.

2.7. Test for flavonoids

2.7.1. Alkaline reagent test

A few drop of sodium hydroxide solution was added to the extract. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

2.8. Test for proteins and amino acids

2.8.1. Biuret test

3ml of test solution was added to 4% sodium hydroxide and few drops of 1% copper sulphate solution. Formation of violet colour indicates the presence of proteins.

2.8.2. Ninhydrin test

A mixture of 3ml test solution and 3 drops of 5% Ninhydrin solution was heated in a boiling water bath for 10min. Formation of purple or bluish colour indicates the presence of free amino acids. Formation of purple or bluish colour indicates the presence of free amino acids.

2.9. Test for tannins

2.9.1. Lead acetate test

A few drops of lead acetate was added to 5ml of aqueous extract. Formation of yellow or red colour precipitate indicates the presence of tannins.[5,6,7,8]

3. Experimental worms

All experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all faecal matters.

4. Administration of Albendazole

Albendazole (100 mg/ml) was prepared by using distilled water and was administered as per method of extract.

5. Anthelmintic Assay

The anthelmintic activity was carried out on adult Indian earthworms, *Pheretima posthuma* as per method with minor modifications.[9] All formulation and standard drug preparations were freshly prepared before starting the experiment. Four groups of approximately equal size earthworms consisting of two earthworms in each group were used for the present study. *Pheretima posthuma* was placed in Petri dish containing two different concentrations (50 &100mg/ml) of formulation and standard (100mg/ml).

Group-1: Control (Normal saline)

Group-2: Standard (Albendazole- 100mg/ml)

Group-3: Formulation (Low Dose-50mg/ml)

Group-4: Formulation (High Dose-100mg/ml)

Observations were made for the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^0C followed with fading of their body colour.[10,11]

6. Statistical Analysis

All the data were expressed as the mean ± standard error of the mean (SEM). The statistical significance of the differences between the groups was analyzed by using GraphPad 5.0 software (GraphPad, San Diego, USA) by applying one way Analysis Of Variance (ANOVA) followed by Tukey's multiple comparison test as post hoc. The values of $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

1. Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the formulation showed the presence of various phytochemical constituents and is depicted in Table-1.

Table 1: Observation Table for Phytochemical Test of Formulation:

Sl. no.	Family of compound	Formulation
1.	Alkaloids	+
2.	Glycosides.	+
3.	Triterpenoids	+
4.	Flavonoids	+
5.	Saponin	+
6.	Carbohydrate	+
7.	Steroids and	+
8.	Protein and amino acids	+
9.	Tannins	+

2. Anthelmintic Activity Assay

Table-2 depicts the time taken for paralysis and death of earthworms after treating with various treatment groups.

Table 2: Anthelmintic activity of the Formulation

Group	Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
I	Control	-	-	-
II	Standard	100	14.41 ± 0.0750^{a}	22.44 ± 0.0900^{a}
III	Formulation (Low Conc.)	50	36.10 ± 0.0350^{adg}	48.84 ± 0.4900^{adg}
IV	Formulation (High Conc.)	100	23.36 ± 0.1850^{adg}	33.42 ± 0.1700^{adg}

Results expressed as Mean ± SEM; (n=2)

[a]P< 0.001, [b]P< 0.01, [c]P< 0.05, when all groups were compared with control;

[d]P< 0.001, [e]P< 0.01, [f]P< 0.05, when all groups were compared with standard;

[g]P< 0.001, [h]P< 0.01, [i]P< 0.05, when formulations were compared with each other.

Results expressed as Mean ± SEM

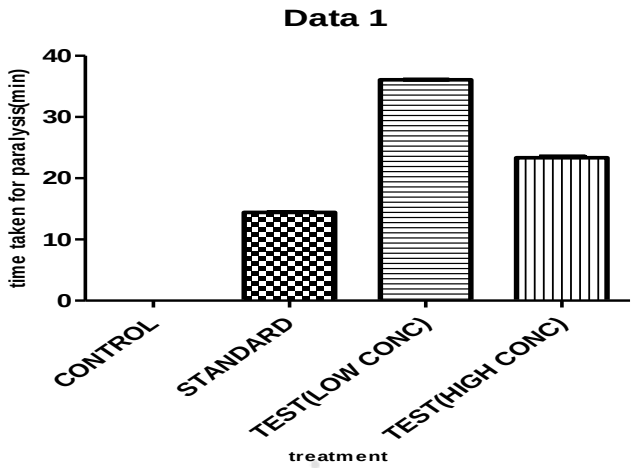

Figure 1: Graphical representation for the anthelmintic activity of different treatment groups (Paralysis)

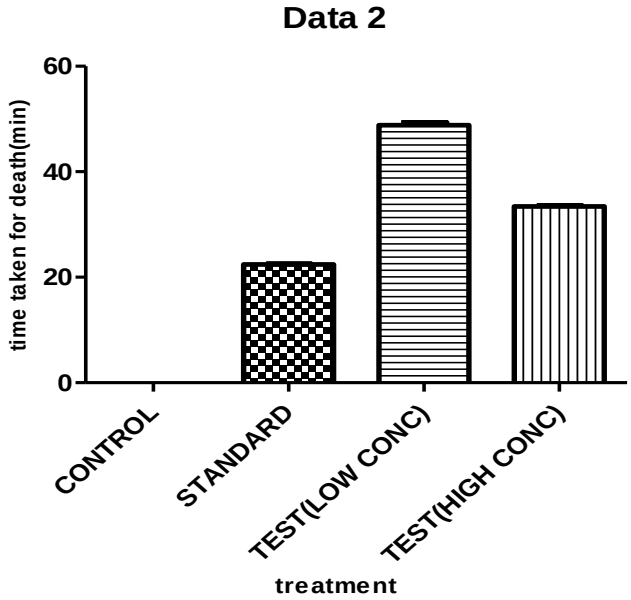

Figure 2; Graphical representation for the anthelmintic activity of different treatment group (death)

CONCLUSION

The aim of the present study was to carry out the preliminary phytochemical screening of Drakshadi kashayam (an Ayurvedic formulation) and to evaluate the anthelmintic activity of the same.

Phytochemical analysis of the formulation was carried out in order to find out various phytochemical constituents present in the preparation, which showed the presence of tannins, flavonoids and saponins as one of the chemical constituents. These constituents were shown to possess anthelminthic activity.[12,13] Tannins are found to bind to free proteins in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite and cause death and this might be the reason for the anthelmintic activity of the formulation.[14]

The anthelmintic activity assay revealed that the formulation possessed a dose dependant anthelmintic activity which was comparable to that of the standard drug. Thus from the study, it can be concluded that Drakshadi kashayam is a potent anthelmintic agent. Further research efforts are required for the depilation of chemical constituent and study of the individual constituents which all are responsible for this activity.

ACKNOWLEDGEMENT

The authors are grateful to thank the management of Prime College of Pharmacy Palakkad, for providing all the facilities to do the research work.

REFFERENCES

1. https://en.wikipedia.org/wiki/Ayurveda.
2. https://draxe.com/ayurvedic-medicine/.
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4127824/.
4. https://en.wikipedia.org/wiki/Anthelmintic.
5. Kirtikar K.R, Basu, B.D. Indian medicinal plants. 2006; II: 936-938.
6. Khandelwal K.R. Textbook of practical pharmacognosy. 11th ed. Pune: Nirali Prakashan. 2004; 149-155.
7. Trease G, Evans W. Textbook of Pharmacognosy, XIII Ed., BaillIere Tindal, London. 1989; 799 – 803.
8. Kokate C.K. Practical Pharmacognosy, IV edition. Nirali Prakashan. 1994; 108-109. (1994).
9. Ajaiyeoba E.O., Onocha P.A., Olorenwaju O.T., *Pharm Biol*, 2001, 39, 217-220.
10. Qureshi M.S. *et al*., Research J. of Pharmacognosy and Phytochemistry, 2009, 1, 78-79.
11. Dwivedi S. *et al.*, Ethno. Leaflets, 12, 741-743.
12. Niezen JH, Waghorn GC, Charleston WA. Growth and gastrointestinal nematode parasitisum in lambs grazing either Lucerne (*Medicago saliva*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J Agric Sci 1995;125:281-9.
13. Pal DK, Sahoo M, Mishra AK. Anthelmenthic activity of stems of *Opuntia vulgaris* mill. Asian J Chem 2007; 19:793-5.

14. Mali RG, Wadekar RR. *In vitro* Anthelmintic activity of *Baliospermum montanum* Muell: Arg roots. Indian J Pharm Sci 2008;70:131-3.