Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 15 (4), 2024; 967-971

Evaluation of Synergistic Anthelmintic Activity of *Citrus Aurantifolia* and *Feronia Limonia* Leaves Juice Extract Using *Pheretima Posthuma* Model

Research Article

Anup Shende[1*], Mrunal Deshmukh[2], Siddhant More[1], Achal Dingalwar[1]

1. Research Scholar, 2. Assistant Professor, Department of Pharmaceutics,
Datta Meghe College of Pharmacy,
Datta Meghe Institute of Higher Education & Research (DU), Wardha. Maharashtra. India.

Abstract

With rising anthelmintic costs and resistance, there's a need for cost-effective alternatives. Synergistic effects of *Citrus aurantifolia* (Christm.) Swingle and *Feronia limonia* (L.) Swingle are unexplored. The prospective anthelmintic effects of *Citrus aurantifolia* (Christm.) Swingle (Acidlime) and *Feronia limonia* (L.) Swingle (kaitha) leaf juice, as well as the combination of leaf juice on *Pheretima Posthuma*, were investigated in the current study using in-vitro studies. The anatomical and physiological characteristics of *Pheretima Posthuma* are similar to intestinal roundworm parasites of human beings hence they are used for in-vitro study. Different concentrations (20% 50%, 100%) of juice of acidlime and kaitha and a combination of juice of both leaves were tested. Results were articulated in terms of time (min) of paralysis and death of worms. The usual medication albendazole is used as a positive control in these experiments, whereas saline water is used as a negative control. Results of Anthelmintic activity were observed in dose-dependent manner. A comparison of combination of juices of acidlime and kaitha leaves at 100% concentrate with Albedazole medication demonstrated a comparable anthelmintic activity. Albendazole (10mg/ml) while individual juice at 100%, 50%, and 20% concentrate showed less significant activity against worm *Pheretima Posthuma*. This study demonstrates the possibility of juice of *kaitha* leaves combined with acidlime leaves as anthelmintic drug. The concentration of a combination of juices showed a better anthelmintic effect when compared to individual juice. The drug's phytochemical profile may be further examined to determine the active ingredient responsible for its anthelmintic effect.

Keywords: Helminthiasis, Anthelmintics, *Citrus aurantifolia,* Woodapple, Synergism, *Pheretima Posthuma*.

Introduction

Around 1.5 billion individuals suffer from helminthiasis, a parasitic helminth infection caused by several types of parasitic helminths (worms) (1). One of the world's biggest public health issues is helminths' parasitic invasion of the body human. About 800 million people are thought to have worm infections of some kind. In certain tropical places, worm infection approaches 90%, and individuals may be afflicted with many forms of helminths (2). Aside from the tropics' unusual environmental situations, poverty, illiteracy, a lack of proper sanitary facilities, and a lack of pure water supply make total eradication extremely difficult (3). Helminthiasis control is performed by the use of synthetic anthelmintics, however, there are some disadvantages, such as the development of resistant populations, high cost, risk of environmental pollution and reduction of animal production due to low effectiveness has awakened interest in medicinal plants as an alternative source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in the control of parasites and anthelmintic drugs (4).

Nemathelminths, or roundworms, are one kind of helminths, whereas platyhelminths are flatworms. The second group is further divided into two groups: cestodes (tapeworms) and trematodes (flukes). Almost 350 helminth species have been discovered in humans, with the majority colonising the gastrointestinal (GI) tract. The clinical effects of helminthiasis vary: for example, threadworm infections are mostly unpleasant, but schistosomiasis (bilharzia) or hookworm infection is linked with significant morbidity. In helminth-infected children, anaemia, nutritional issues, and cognitive impairment are prevalent. Helminthiasis is frequently co-endemic with malaria, TB, and HIV/AIDS, hence increasing the disease burden and interfering with vaccine programs (1). Intestinal Roundworms (*Ascaris lumbricoides, Enterobius vermicularis*), hookworms, threadworms, tapeworms (*Taenia saginata, Taenia solium*), filarial worms or tissue roundworms, and schistosomes are the most often detected parasites (1) (3). The global range and occurrence of helminthiasis have been reviewed by Lustigman et al. (2012) (1). The majority of helminthic infections cause modest

*** Corresponding Author:**
Anup Shende
Research Scholar,
Datta Meghe College of Pharmacy, Datta Meghe Institute of Higher Education & Research (DU), Wardha 442001, Maharashtra India.
Email Id: anup.s.shende@gmail.com

symptoms or debility, while some, such as Schistosoma species, can cause considerable morbidity. Even though worms have rather complicated life cycles, most medication treatment is geared against adult worms found in the human gut or extraluminal tissues (5).

Anthelmintics are medications used to treat helminthiasis. In endemic locations anthelmintic therapy's main objective should be to reduce infection rates below the level that is considered clinically relevant. Often, a thorough parasitological treatment is not achievable (3). The majority of anthelmintic agents act on the helminths in such a way that peristaltic action or catharsis expels the parasites from the intestinal system. Anthelmintic medicine may not kill the worms, but this is unimportant if the infestation is eradicated (2). A vermicidal anthelmintic medicine kills the worm, whereas a Vermifuge anthelmintic drug enables the worm's simple ejection without killing it (3). Anthelmintics of botanical origin represent a viable alternative for managing parasitic infections. Investigations within the domain of phytomedicine serve as a valuable reservoir of insights concerning the plausible therapeutic efficacy of plant extracts against specific ailments and pestilences. As a result, this area of study has witnessed impressive development related to human and animal health (4). Herbal medications have been used to treat parasite infections in humans since ancient times and may be useful in reducing the development of resistance (6). There are reports indicating antiparasitic effects of some plant species, such as *Citrus aurantifolia*, *Piper tuberculatum, Lippia sidoides, Mentha piperita, Hura crepitans,* and *Carapa guianensis* (4). Herbal medications have been used for the treatment of parasite infections in humans since ancient times and may be useful in reducing the development of resistance (6). Many underutilised medicinal plants that can treat a variety of human diseases and ailments may be found in abundance in India (7). Medical practitioners widely use synthetic drugs or modern medicine for treating various diseases (8),(9). However, side effects accompany this system of medicine and it is not economical. Thus, people have started to take interest in herbal medicine/green medicine since they are easily available, economical, and have minimal adverse effects or no harmful effects on the human body (10).

The herb *Citrus aurantifolia* grows annually. The herb is mostly utilised as an anthelmintic and antioxidant. It is used to treat skin issues, ulcers, and conjunctivitis by reducing inflammation. The herb works well for vomiting and dyspepsia (11). The fruits of *Citrus aurantifolia* are distinguished for their acidic taste and elevated content of vitamin C, recognized for its immunomodulatory effects, antioxidative properties, and capacity to mitigate oxidative stress. A decoction derived from the bark or rind of the fruit is employed in the management of colic. *Citrus aurantifolia* exhibits notable antiseptic, antibacterial, and antiviral attributes. While its anthelmintic potential is acknowledged, empirical validation is pending. As a result, the aim pf the current study was to assess the in-vitro anthelmintic efficacy of several solvents extracts of *Citrus aurantifolia* (8). *Citrus aurantifolia* fruit empty juice sacs were tested for anthelmintic action. The initial qualitative phytochemical screening of the peel of *Citrus aurantifolia* revealed the presence of steroids, terpenoids, flavonoids, and phenolic compounds. These phytochemicals are in charge of plants' biological function. This investigation tested the anthelmintic activity of peel extracts based on their diverse biological activities. According to the current account is the first study on the anthelmintic properties of methanol, ethanol, and citrus extracts. In the Indian state of Assam, the Biswanath region produced acid lime nonripe peel (6).

Kaitha (*Feronia Limonia L.*) is a significant plant that is a member of the Rutaceae family and has a range of therapeutic and nutritional uses. Scientific investigations have proven its ethnomedicinal capabilities as well as several bioactive chemicals found in various regions of *kaitha*. In Ayurvedic medicine, the leaves, bark, roots, fruits, and seeds are frequently used as a laxative, to treat a range of illnesses, and to treat peptic ulcers, dysentery, and chronic diarrhoea. The fruit pulp also has free radical scavenging, lipid peroxidation inhibition, antibacterial, antioxidant, antiviral, anti-diarrheal, gastro-protective, antidiabetic, anti-ulcerative colitis, hepatoprotective, cardioprotective, and radioprotective activities (7).

The primary aim of this study is to evaluate the synergistic anthelmintic activity of *Citrus aurantifolia* (Acidlime) and *Feronia limonia* (Kaitha) leaf juice extracts using the *Pheretima posthuma* model. The specific objectives of the study are, To evaluate the anthelmintic activity of *Citrus aurantifolia* as well as *Feronia limonia* leaf juice at different concentrations (20%, 50%, 100%). To investigate the synergistic effects of the combined juices of *Citrus aurantifolia* and *Feronia limonia* on *Pheretima posthuma.* To compare the efficacy of the combined leaf juice extracts with the standard anthelmintic drug, Albendazole.

Materials and Methods

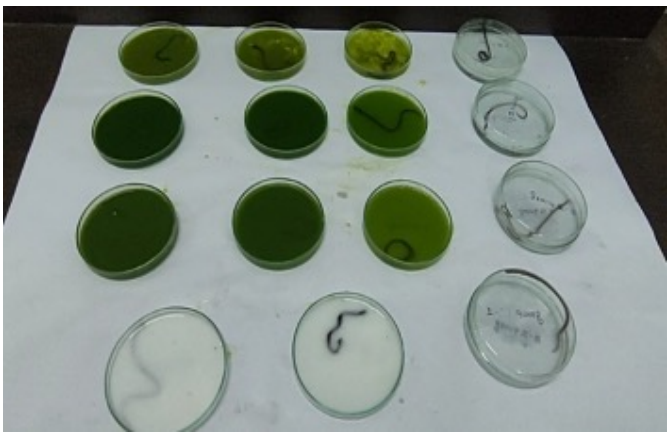
Collection of Worms

The Indian earthworm *Pheretima Posthuma* specimens were procured from the Centre of Science for Villages, Dattapur, Wardha, for scientific investigation. These adult specimens of *Pheretima Posthuma* were utilised for the evaluation of anthelmintic activity. The earthworms were retrieved from the vermicompost tank located at the Center of Science for Villages, Dattapur, Wardha, and subsequently subjected to a cleansing procedure to eliminate extraneous matter. All experimental procedures were conducted on earthworms measuring 8-14 cm in length and 0.2-0.3 cm in width. Because earthworms are easily found in fields and help to increase the fertility of the soil, they are similar to *A. lumbricoides*, a parasite that causes intestinal roundworms in humans both physically and physiologically. Therefore, earthworms have been selected as an experimental animal for the preliminary evaluation of anthelmintic activity, in vitro (12) .

Plant Collection and Authentication

Acid lime and *kaitha* leaves were picked up from the Mahatma Gandhi Ayurved College Hospital and Research Center Salod (H.), Wardha. and authenticated by the Department of Fruit Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Inward no. No HDFS/219 Dt 20/05/2022.

Figure 1: Leaves Juice Extract Using *Pheretima Posthuma* Model



Preparation of Juice

The fresh leaves of Acid lime and *kaitha* leaves were picked up from the Mahatma Gandhi Ayurved College Hospital and Research Center Salod (H.), Wardha. and identified in the department of Pharmacognosy at Mahatma Gandhi Ayurved College Hospital and Research Center Salod (H.), Wardha. The leaves were properly cleaned to eliminate any adherent residue and grind thoroughly in the mixer. The juice had been filtered. No. 40 Whatman filter paper The filter-out was collected. The juice was diluted with the help of distilled water in the following concentrations: 100 percent concentration, 50 percent concentration, and 20 percent concentration.

Administration of Albendazole

Albendazole oral suspension I.P. is used as the standard drug. Albendazole (40 mg/ml).

Experimental Design

The anthelmintic assay was conducted following the methodology outlined by (13), with slight modifications by (14). Adult specimens of Indian earthworms (*Pheretima Posthuma*) measuring between 8 to 14 cm in length and 0.2 to 0.3 cm in width were utilised. The specimens were divided into four cohorts, each comprising four earthworms. One earthworm in each cohort served as the control and was immersed in distilled water. Solutions of varying concentrations (100%, 50%, and 20% in distilled water) were prepared in amounts of 30ml. Paralysis onset was determined by vigorously agitating the earthworms, noting the absence of movement. Death was ascertained when the earthworms ceased movement even upon vigorous agitation or immersion in warm water [50^{o}C].

Statistical Analysis

Table 1: Anthelmintic activity of herbs in different concentrations

Sr. No	Test substance	Concentration	Time for paralysis (min)	Time for death (min)
1	*Feronia Limonia* (Kaitha)	20%	113.8833 ± 1.579135	262.4833 ± 1.45522
		50%	87.41667 ± 1.020621	227.8333 ± 1.600833
		100%	38.66667 ± 1.632993	119.6667 ± 1.505545
2	*Citrus aurantifolia* (Acidlime)	20%	43.31667 ± 0.752108	86.23333 ± 0.814043
		50%	26.05 ± 0.656506	45.96667 ± 0.722957
		100%	14.86667 ± 1.304863	32.8 ± 1.017841
3	Combination Of *Feronia Limonia*(Kaitha) + *Citrus Aurantifolia* (Acidlime)	20%	41.53333 ± 0.922316	102.3833 ± 0.806019
		50%	26.11667 ± 0.716705	45.43333 ± 0.739369
		100%	15.81667 ± 0.832867	34.38333 ± 1.000833
4	Positive Control	40mg/mL	6.616667 ± 0.402078	27.06667 ± 0.581951

Each value represents mean±SEM (N=6)

Results

As table no 1. shows various concentrations of the leaves' Juices of Acid lime and *kaitha*. In three types of concentration 100% 50% and 20%. It also includes the combination of two leaves juices which shows synergistic activity of the juices on the worms It shows the shortest time of paralysed and death of the worms as 100% (P=15.81667 ± 0.832867 min & D=34.38333 ± 1.000833 min), 50% (P=26.11667 ± 0.716705 min & D=45.43333 ± 0.739369 min) and 20% (P=41.53333 ± 0.922316 min & D=102.3833 ± 0.806019 min). *Feronia limonia* showed less activity than of *Citrus aurantifolia* and a combination of both *Feronia limonia* leaves but in combination of leaves juice, it shows potent Synergistic activity on the worms. *Feronia limonia* showed reading as 100% (P=38.66667 ± 1.632993min & D=119.6667 ± 1.505545 min), 50% (P=87.41667 ± 1.020621 min & D=227.8333 ± 1.600833min) and 20% (P=113.8833 ± 1.579135 min & D=262.4833 ± 1.45522 min). *Citrus aurantifolia* shows potent anthelmintic activity as 100% (P=14.86667 ± 1.304863min & D=32.8 ± 1.01784 min), 50% (P=26.05 ± 0.656506 min & D=45.96667 ± 0.722957 min) and 20% (P=43.31667 ± 0.752108 min

& D=86.23333 ± 0.814043 min). The standard drug Albendazole shows the same effect at P = 6.616667 ± 0.402078 & D =27.06667 ± 0.581951. Thus, Combination of acid lime and *kaitha* juices of leaves showed significant Anthelmintic activity as compared to standard reference and control.

Discussion

Anthelmintic efficacy has been observed in the foliage of Acid lime and *kaitha*. The paralysis onset time (P) and time to mortality (D) for *Pheretima Posthuma* worms were documented in Table 1. *Pheretima Posthuma*, a mature Indian oligochaete, was selected for experimentation due to its anatomical and physiological resemblance to the human intestinal nematode parasite. Oligochaetes have frequently been utilised for initial in vitro evaluation of anthelmintic botanicals owing to their accessibility. The preliminary phytochemical analysis of an aqueous Acid lime L leaf extract indicated the presence of alkaloids, tannins, proteins, carbohydrates, phenolic compounds, oils, lipids, and flavonoids, which predominantly contribute to the anthelmintic activity investigated in this study., Acid lime aqueous leaf extract caused worms to become paralysed as well as die (11). This research makes it quite clear that *kaitha* is a significant medicinal herb. Numerous phytochemicals are present, and they are what give this plant its therapeutic significance. All of *kaitha's* principal components, including the leaf, fruit, seed, bark, and root, have a wide range of biological activity and are used to treat a wide range of illnesses (7).

The present study was on the anthelmintic activity of *Citrus aurantifolia* and *Feronia limonia* leaf juices, both individually and in combination, using the *Pheretima Posthuma* model. Significant anthelmintic activity was found in the results, much more when extracts were combined, indicating possible synergistic effects.

Results show that the extracts of *Citrus aurantifolia* and *Feronia limonia* have intrinsic anthelmintic activity, thus justifying the folk-ethical uses of these plants. This is, then, indication of a synergistic interaction of the bioactive compounds of the two plants since their combination showed better potential than the individual extracts. This synergism may result from the combined action of flavonoids, alkaloids, tannins, and phenolic compounds, which were reported with positive anthelmintic properties.

Previous studies have reported the anthelmintic potential of various plant extracts. For instance, a study by (15) demonstrated the anthelmintic activity of *Citrus aurantifolia* fruit peel extracts against earthworms, which supports our findings regarding the efficacy *of Citrus aurantifolia*. Similarly, research by (16) highlighted the anthelmintic properties of *Feronia limonia* fruit pulp, corroborating our results with *Feronia limonia* leaf extracts.

However, our study is unique in that it explores the combination of these two plant extracts. The enhanced efficacy observed in the combination could be due to the complementary actions of different phytochemicals, which may target multiple sites on the parasites or enhance each other's bioavailability.

The variations in activity of single and combined extracts against helminths may be due to various factors.

- Phytochemical Interaction: The synergistic effects could be produced from the interaction of various phytochemicals present in the extracts, and it could be that which is causing the increase in overall efficacy.
- Concentration Variations: The varying concentrations of the extracts might modulate the bioavailability and potency of active compounds and hence increase the effect when combined.
- Methodological Differences: Variations in methods of extraction, experimental models, and assessment criteria may be responsible for the differences found.

While preliminary findings indicate the promising anthelmintic properties exhibited through mixing the leaf juices of *Citrus aurantifolia* and *Feronia limonia*, much is left unknown regarding the underlying mechanisms. Further investigation aims to identify and scrutinise the distinctive ingredients accountable for the noticeable impacts. Additionally, undertaking living organism experiments and regulated medical analyses would be paramount to authenticate the effectiveness and risk-free application of these botanical extracts for combating parasitic worm infections among people. Considering the positive signs presented, continued exploration into optimising concentrations and isolation of the bioactive constituents remains scientifically warranted and potentially possesses significant practical value.

Conclusion

The anthelmintic activity of *Citrus aurantifolia* and *Feronia limonia* is attributed to the presence of flavonoids, alkaloids, tannins, and phenolic compounds, validating its traditional application in the treatment of Helminthiasis. The data are presented as mean ± SEM (N = 6). Based on the above-mentioned findings, it is deduced that all aqueous extracts derived from the leaves of *Citrus aurantifolia* and *Feronia limonia* exhibit significant anthelmintic activity The concentration of a combination of juices showed a better anthelmintic effect when compared to individual juice. The drug's phytochemical profile may be further examined to determine the active ingredient responsible for its anthelmintic effect.

Acknowledgment

The authors are grateful to the Department Of Fruit Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for providing the authentication required for the research work. Thanks To Central Pre-Clinical Research Facility (Animal House) Datta Meghe Institute Of Higher Education And Research, Wardha for providing Laboratory facilities to carry out this research activity.

References

1. Ritter JM, MacEwan D, Flower R, Robinson E, Henderson G, Fullerton J, et al. Rang and Dale's pharmacology. Tenth edition. Rang HP, Dale MM, editors. London New York Oxford: Elsevier; 2024. 850 p.
2. The Pharmacologic Principles of Medical Practice: A Text-book on Pharmacology and Therapeutics for Medical Students, Physicians, and the Members of the Professions Allied to Medicine. JAMA. 1958 Apr 12;166(15):1918.
3. Satoskar RS, Rege NN, Bhandarkar SD. Pharmacology and Pharmacotherapeutics. 26th edition. Elsevier India; 2020.
4. Carvalho CO, Chagas ACS, Cotinguiba F, Furlan M, Brito LG, Chaves FCM, et al. The anthelmintic effect of plant extracts on Haemonchus contortus and Strongyloides venezuelensis. Veterinary Parasitology. 2012 Feb;183(3–4):260–8.
5. Lancaster R. Pharmacology in Clinical Practice. First edition. Kent: Elsevier Science; 2013. 322 p.
6. Upadhyaya S. Citrus limon L Burmf peel: Potential anthelmintic agent against Indian Earthworm Eicinia foetida. J Drug Delivery Ther. 2018 Sep 10;8(5):248–50.
7. Srivastava R, Mishra N, Agarwal S, Mishra N. Pharmacological and Phytochemical Properties of Kaitha (Feronia limonia L.): A Review. Int J Pharm Sci Res. 2019;10(3):1-15.
8. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris; IJCM. 2015;06(09):635–42.
9. Li FS, Weng JK. Demystifying traditional herbal medicine with modern approach. Nature Plants. 2017 Jul 31;3(8):17109.
10. Patwardhan B, Vaidya ADB, Chorghade M. Ayurveda and natural products drug discovery. Curr Sci. 2004;86(6):789-799
11. Sravanthi M, Swetha G, Maheshwari A, Shravya K, Harika B, Nureen, Raju K. In-vitro anthelmintic activity of Citrus limon leaf and its phytochemical investigation. J Pharmacogn Phytochem. 2020;9(3):816-818 .
12. Aryal A. Evaluation of Anthelmintic Activity of Citrus reticulata: In Vitro and Its Phytochemical Investigation. Asian J Pharm Clin Res. 2017;10(5):278
13. Mali R, Mahajan SG, Mehta A. In-vitro anthelmintic activity of stem bark of Mimusops elengi Linn. Pharmacogn Mag. 2007;3:73.
14. Mute V.M., Sampat V.M., Patel K.A., Sanghavi K., Mirchandani D., Babaria P.C. Anthelmintic effect of Tamarind indica Linn leaves juice extract on Pheretima posthuma. Int. J. Pharm. Res. Dev. 2009;7:1-6.
15. Abdelqader A, Qarallah B, AL-Ramamneh D, Daş G. Anthelmintic effects of citrus peels ethanolic extracts against Ascaridia galli. Veterinary parasitology. 2012 Mar 13;188:78–84.
16. Tenguria PS and R. Phytochemical Properties and Health Benefits of Limonia acidissima: A Review. International Research Journal of Plant Science. 2021 Jun 8;1–6.

*****