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Research Article

Evaluation Of Anthelmintic Efficacy of Poly Herbal Formulation Specifically Tablets Against Indian Earthworm

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ABSTRACT

Helminthiasis is a widespread parasitic infection among humans and animals caused by helminths. The disease is highly prevalent particularly in developing countries due to inadequate sanitary conditions and poor management practices. The present research work is based on the formulation of poly herbal granules by incorporating the leaves extract of Curcuma longa, Moringa oleifera and Murraya koenigii. These plants are traditionally recognized for their medicinal properties, particularly their effectiveness against parasitic worm infections. The granules was prepared using aqueous extracts of the selected plants, aiming to provide a synergistic effect for enhanced anthelmintic activity. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as alkaloids, tannins, saponins, and flavonoids, which are known to exhibit anthelmintic potential. In vitro studies using Pheretima posthuma (Indian earthworm) demonstrated significant paralysis and death times, indicating strong wormicidal activity. The formulated granules was also evaluated for physicochemical parameters and stability. Results suggest that the polyherbal combination can serve as an effective and natural alternative to synthetic anthelmintic drugs with reduced side effects.

INTRODUCTION

Medicinal plants are a crucial component in traditional and modern healthcare. Curry leaves (Murraya koenigii) are prized in Asian cuisine and recognized for their array of bioactive compounds

and pharmacological significance. ^[1] Plants have been indispensable to human survival for millennia, serving as vital resources for food, clothing, and shelter. Their components have been globally utilized in medicine, food preservation, crafts, cosmetics, essential oils, flavouring, scented products, and dietary supplements. ^[2]

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Herbal medicine is estimated to be the primary healthcare provider for 80% of the population in Asian and African nations. Pharmaceutical companies are increasingly focusing on plant-based medications, and many active constituents have been isolated and evaluated for their pharmacological activities. Digestive disorders are common and can cause various symptoms, but natural remedies like the digestive polyherbal tablet, formulated with natural ingredients used for centuries to improve digestion, can aid in digestive health. [3] Turmeric, derived from the rhizomes of *Curcuma longa*, is a perennial plant having short stem with large oblong leaves, and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in color. Accounting for about 78 percent of world turmeric production, India is the largest producer of turmeric. [4] *Murraya koenigii* (curry leaf, kari patta) is a member of the Rutaceae family. Traditionally, it is utilized as an antiemetic, antidiarrheal, febrifuge, blood purifier, tonic, and flavoring agent in curries and chutneys. Its essential oil is applied externally for bruises and eruptions and finds uses in the cosmetic and perfume industries. The plant, typically a small shrub up to 6m, emits a pleasant aroma and contains a variety of chemical compounds that provide biological and pharmacological benefits. [5] The therapeutic value of curry leaves is attributed to a rich suite of bioactive compounds—alkaloids, flavonoids, and essential oils—which account for its antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective effects. These phytochemicals also underlie its traditional use for treating an array of health issues, from digestive and metabolic disorders to skin problems. [5] *Moringa oleifera*, Lam syn. *M. pterygosperma*, Gaertn (Family – Moringaceae), is native to India and the entire plant has been used to treat various ailments. The leaves have enormous properties. They are antibacterial, antifungal, Antipyretic,

wound healing, Antiasthmatic, Hepatoprotective, Antithyroid, Radioprotective, Antiulcer, Antihyperglycemic, Antitumor, Ant plasmodial, antioxidant activity, Prophylactic activity, analgesic, antifertility, anticonvulsant and antilipidemic. *Moringa oleifera* is reported to contain a wide range of phytochemicals that are responsible for its activities. [6] Helminths are parasitic worms that can infect humans and other animals. There are different types of helminths: flukes (trematodes), tapeworms (cestodes), roundworms (nematodes) and thorny-headed worms. When these worms enter into the human body, they cause parasitic infection, which can appear as a intestinal worms or lung flukes. This infection is known as helminthiasis, although it's sometimes called helminthosis or simply a worm infection. An Anthelmintic is a drug used to treat infection caused by parasitic worms also known as helminthes. [7] Helminthiasis causes a significant health problem with increased morbidity and, to some extent, mortality in an underdeveloped and developing country, although it may also occur in developed countries. [7] Helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, pneumonia, eosinophilia and anemia, eosinophilia and pneumonia. Worldwide prevalence lies between 500 million to one billion annually approximately. A medication that eliminates or kills gastrointestinal worms is called an anthelmintic. The terms "wormer" and "dewormer" are more widely used. Other names for anthelmintics include nematocides, parasitics, endectocides, parasiticides, and drenches. They can also be called vermifuges (astonishing) or vermicides (killing). [8] Anthelmintic drugs target the helminth parasitic worms and expel them from the body, either by stunning or by killing them. Ideally an anthelmintic agent should have a broad spectrum of action, high percentage of cure,



free from toxicity to the host and should be cost effective, but one of the synthetic drugs available in the market meets these requirements. Moreover as helminthes are increasingly becoming resistant to classical drugs, there is an urgent need for search and development of new antihelminthes agent, preferably with novel mode of action. Even the most common drugs like piperazine salt have been shown to have side effects like nausea, intestinal disturbance and giddiness and the high cost of modern antihelminthics has limited the effective control of the parasites. This leads to renewed interest in screening of medicinal plants for their antihelminthic activity. The traditional medicines hold a great promise as source of easily available effective antihelminthic agents to the people, particularly in tropical developing countries. [9] Herbal remedies are a better option because they are nontoxic and profitable, which greatly stimulates the anthelmintic activity of medicinal herbs. This study aimed to comprehensively determine the antihelminthic activity of some indigenous plants found in India, The purpose of the present study was to prepare a poly herbal formulations contains natural ingredients such as turmeric, moringa and , curry leaf which have been traditionally used for their digestive properties. Further, the aim of this study, is to evaluate the physicochemical and pharmacological properties of the poly-herbal granules derived from the folk recipe containing turmeric, moringa and , curry leaf by using simple conventional techniques.

MATERIALS AND METHODS

The experiment was conducted at the Santoshi Institute of Pharmaceutical Education and Research during August 2025 to assess the anthelmintic activity against worms. Albendazole, Normal saline were purchased from authorized pharmaceuticals. The solvents and other chemicals

used during experimental protocol were of analytical grade.

Plant materials and preparation of test substances

The leaves of three species: turmeric, moringa and , curry leaf were collected from different areas like Baripada, Mayurbhanj and Jajpur. The identification and authentication process was conducted by Dr. Durga Prasad Barik, Ravenshaw University, a botanist affiliated with Ravenshaw University, Odisha The authenticated specimens have been preserved as vouchers for future reference. These leaves were ground into fine particles and combined in equal ratios. A total of approximately 500 grams of the mixed plant powder was subjected to extraction using ethanol as the solvent, employing the hot extraction method with soxhlet equipment. The extraction continued until the solvent in the thimble became clear, indicating the completion of the process. Finally, the resulting extract was evaporated to dryness using a vacuum desiccator.

Phytochemical investigation of the extract

Preliminary qualitative phytochemical investigation was carried out to identify the active chemical constituents present in the extracts of leaves of. Flavonoids were tested using the ferric chloride test and the lead acetate solution test. The evaluation of proteins was performed through the Xanthoprotein test and the ninhydrine test. Amino acids were assessed with the ninhydrine test and the tyrosine test. Furthermore, phenols were analysed via the ferric chloride test, and tests for organic acids were also carried out.^[10]

Preparation of polyherbal tablet by adding excipients



The polyherbal preparation specifically tablets containing turmeric, moringa and , curry leaf were prepared using the wet granulation method. Excipients like lactose, microcrystalline cellulose (MCC), magnesium stearate, and maize starch were used. A binder solution was prepared using water and maize starch, and all the ingredients were mixed thoroughly for 15 minutes. The wet mass was passed through a sieve and dried in a tray drying oven. Finally, the powder blend was mixed with magnesium stearate and compressed into a 250 mg tablet using a multi station tablet press. ^[11]

Worm collection and authentication

Indian earthworms *Pheretima posthuma* (Annelida) were collected from the water logged areas. They were washed with tap water for the removal of the adhering dirt. Aquarium worms *Tubifex tubifex* (Annelida) were collected from the local market.

Qualitative Phytochemical Analysis

Qualitative assay of the Poly Herbal Formulation for the presence of phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins etc were performed following Standard procedure. ^[12]

Glycosides: For glycosides 1 mL of freshly prepared 10% KOH was added to 1 mL of Poly Herbal Formulation. The presence of glycosides was confirmed by the formation of brick red precipitates.

Saponins: For saponins, frothing test was performed in which 2 ml of the Poly Herbal Formulation was vigorously shaken in the test tube for 2 minutes. Presence of frothing indicated saponins.

Steroids: Steroids were identified by adding 5 drops of concentrated H₂SO₄ to 1 mL of the Poly

Herbal Formulation in a test tube. Red coloration indicated the presence of steroids.

Triterpenes: For triterpenes, 5 drops of concentrated H₂SO₄ were added to 1 mL of Poly Herbal Formulation. Appearance of blue green colour indicated the presence of triterpenes.

Flavonoids: Presence of flavonoids was tested by adding 1 mL of freshly prepared 5% AlCl₃ solution to 1 mL of Poly Herbal Formulation. Yellow coloration indicated the presence of flavonoids.

Phenolics: For phenolics, two drops of 5% FeCl₃ were added to 1 mL of the Poly Herbal Formulation in a test tube. Presence of greenish precipitate indicated the presence of phenolics.

Alkaloids: To detect the presence of alkaloids 0.2 gm of Poly Herbal Formulation was warmed with 2% sulphuric acid in a test tube for 2 minutes. The mixture was filtered in a separate test tube and few drops of Dragendroff reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

Statistical application

The experiments were carried out in triplicate. All the results are reported as mean $\pm$ standard deviation (SD).

Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajayieoba et al. with minor modifications. The assay was performed in vitro using adult earthworm (*Pheretima posthuma* and *Tubifex tubifex*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity. ^[12]



The 20 ml formulations containing different concentrations of the polyherbal formulations (50, 75 and 100 mg/ml in distilled water) were prepared and five worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except 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°C. Albendazole (25 mg/ml) was used as reference standard while distilled water as the control.

RESULTS AND DISCUSSION

Plants and its extracts were practiced in traditional and ethnic medicine for the treatment of many diseases such as malaria, dengue fever, wound healing, viral and bacterial diseases, cancer, arthritis, diabetes, menstrual disorders and as supplementary food for normal metabolism and growth of humans. [12] According to the world health organization infectious diseases are the main cause of death and the key agents of the afflicting world wide. These infectious are usually transmitted through contaminated water /food, unwashed hands, feces or contact with contaminated objects. [12] Helminths infections are among the most wide spread infections in humans especially in poverty - stricken and developing countries with warm moist environments and poor sanitary conditions. [12] Worldhealth organization estimates about 2 million people throughout the world affected with parasitic worm infection and the main reason is associated with poor management and inadequate control measures. [13] Whilst, numerous efforts has been made to understand the transmission mode and therapeutic probabilities against Helminthiasis in last few decades, still no prominent product has been established to control specific helminthic infestations. In India, occurrence of these infection

is very high in wet seasons (as high as 100%), the drugs available in market is of high cost and limited effective control over parasitic infections. Plant products are frequently considered to be less toxic and significantly free from side effects than synthetic ones. [13] The present investigation was carried out to understand effectiveness of herbal formulation containing *Curcuma longa* rhizome, leaves of *moringa* and *Curry* leaves in combination against Indian earth worm, *Pheretima posthuma* respectively. The phytochemical screening of poly herbal formulations showed the presence of all major phytoconstituents like alkaloids, flavonoids, glycosides, steroids, terpenoids and phenolic compounds. The preliminary phytochemical analysis of different extracts and poly herbal formulations evidenced the presence of multiple components in it. The results revealed the presence of flavonoids, tannins, steroids, saponins, terpenes, and phenolic compounds in Table 1.

The results of anthelmintic activity of all the poly herbal formulations are summarized in Table 2. It is evident that *Curcuma longa* rhizomes along with *moringa* and *curry* leaves was exhibited dose dependent anthelminthic activity that caused paralysis tested against Albendazole as standard. From the results, it is observed that the herbal formulation showed potent anthelminthic activity, than individual as tested individually the activity result revealed dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death from starting dose of 25 mg/ml to 100 mg/ml respectively. Further it was also observed that the anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 75 mg/ml and 100 mg/ml concentration. The formulations at 100 mg/ml caused paralysis and death for the worms *Pheretima posthuma* (Annelida) and *Tubifex tubifex* respectively. For



Pheretima posthuma the times are 9.6 and 10.5 min as per paralysis is concerned where as the time of death is 16.5 and 15.8 min., while the PHF at 50 and 75 mg/ml of the worm *Pheretima posthuma* is 26.3 and 12.1 min as per paralyzed and as per death it is 38.7 and 21.4 min and *Tubifex tubifex* is 27.6 and 13.4 min as per paralyzed and as per death it is 36.4 and 20.3 min. All the doses showed different anthelmintic activity in terms of mortality rate at the same concentration during the six hours time period. The standard drug (Albendazole) shows paralysis within 10.4 ± 3.12 min and time of death 17.2 ± 4.3 min. All the test groups showed a statistically significant ($p < 0.05$) anthelmintic activity as compared with control and standard group respectively. The observation of the result showed the poly herbal formulation as more potent anthelmintic agent and sensitivity of earthworms increased by increasing dosage of the formulation.

The results can be considered significant since the polyherbal formulation with a number of compounds and can be a source of phytochemicals with anthelmintic activity comparable to standard drugs used. Although the rate of death of worms after each hour was different for different doses, at the end of six hour time period the rate of death of worms was same. A number of studies are available for anthelmintic activity of tannins, alkaloids and flavonoids. Potential anthelmintic activity of plant extracts may due to the presence of tannins in high concentration.^[14] Tannins are able to disturb the metabolism of *Ascaris* species through oxidative phosphorylation reaction.^[15] In addition, tannins can also bind to free protein nutrition which leads to larval starvation. Flavonoids can interact with the free proteins of gastrointestinal host or glycoproteins on the worm's cuticula and blocking tubulin polymerization leading to death of worm.^[16] Saponins causes vacuolization and disintegration

of the tegmental worm through changing their cell membrane permeability. Glycosides and steroids are antioxidant agents that decrease nitrate production; it leads to the inhibition of worm development.^[17]

The mechanism of action of natural anthelmintic agents also involves inhibition of glucose uptake system that causing the loss of energy of worm. Breaking of mucopolysaccharide membrane structure of helminths will restrict their movement which may cause paralysis and finally the worm's death.^[18] Phytochemicals are also involved in blocking of attachment of the parasitic worm to host by damaging of worm's tegumental surface.^[19] The effects also involve the change of phosphatase enzymes in the tegument of parasites.^[20]

CONCLUSION

Plants are one of the most important sources of medicines. The role of medicinal plants in promoting the ability of human health to cope with the unpleasant and difficult situations is well documented from ancient times till date all over the world. Medicinal plants are rich in secondary metabolites which are potential sources of drugs and of therapeutic importance. The present study was conducted to unravel the *in vitro* anthelmintic activity of herbal formulation containing rhizomes of *Curcuma longa* rhizomes along with *moringa* and *curry* leaves mixture in equal quantity. The examined methanolic extract of herbal formulation exhibited higher anthelmintic activity for both estimated parameters of paralysis and death. With these baseline information, a novel formulation can be prepared as new pharmaceutical drug for the treatment and curing of Helminthiasis. This justifies us the use of present combination used in the kitchen for various dishes including as potent anthelmintic agents. Still and all, further



panoramic pharmacological and chemical investigations should be carried out to isolate the active constituents and elucidate appropriate mechanism of action for development of new herbal product.

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Table 1: Preliminary qualitative phytochemical analysis of different extracts and poly herbal formulation

Test for ↓	<i>Murraya koenigii</i>	<i>Curcuma longa</i> rhizomes	<i>Moringa oleifera</i>	PHF (Poly Herbal Formulations)
Tanins	+	+	+	+
Saponins	+	+	-	+
Fats and Oils	-	-	-	-
Alkaloids	+	+	+	+
Flavonoids	+	-	-	+
Coumarin	-	-	-	-
Terpenoids	+	+	-	+
Steroids	+	-	-	+
Phenols	+	-	-	+
Glycosides	+	-	-	+

(+) shows the presence of constituents, (-) shows the absence of constituents.

Table 2: Effect of different doses of Poly Herbal Formulations on anthelmintic activity on *Pheretima Posthuma* and *Tubifex* worms

Group	Treatment	Concentration (mg/ml)	Time taken for paralysis (P) and death (D) of worms in minutes	
			P	D
I	Vehicle	DW	-	-
II	Albendazole	25	10.4 ± 3.12 ^c	17.2 ± 4.3 ^c
anthelmintic activity on <i>Pheretima Posthuma</i>				
III	PHF	50	26.3 ± 1.4	38.7 ± 3.4
IV		75	12.1 ± 1.6 ^c	21.4 ± 3.3 ^c
V		100	9.6 ± 2.4 ^c	16.5 ± 4.2 ^c
		anthelmintic activity on <i>Tubifex</i> worms		
VI		50	27.6 ± 2.4 ^c	36.4 ± 3.4 ^c
VII		75	13.4 ± 4.2 ^c	20.3 ± 4.2 ^c
VIII		100	10.5 ± 3.8 ^c	15.8 ± 4.6 ^c



Values are expressed in MEAN $\pm$ S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at * $p < 0.05$, ** $p < 0.01$) (t-value denotes statistical significance at ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$ respectively, in comparison to group-II).