FORMULATION AND EVALUATION OF *MANGIFERA INDICA* SEED HERBAL SYRUP FOR ITS ANTHELMINTIC ACTIVITY

J Joysa Ruby[1*], Hemalatha K [2], Venkatesh DP[3], K. Selvakumar[4],

Dinesh Mahato[5], Desai Sonali[6]

Department of Pharmaceutics, Acharya & BM Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta Main Road, Bengaluru-560107, Karnataka

[1,3,5,6] Department of Pharmaceutics, Acharya & BM Reddy College of Pharmacy, Rajiv Gandhi University of Health Science, Bengaluru, Karnataka, India.

[2] Department of Pharmacognosy and Phytochemistry, Acharya & BM Reddy College of Pharmacy, Rajiv Gandhi University of Health Science, Bengaluru, Karnataka, India.

[4] Department of Pharmaceutical Quality Assurance, Acharya & BM Reddy College of Pharmacy, Rajiv Gandhi University of Health Science, Bengaluru, Karnataka, India.

***Corresponding author:**

Dr. Joysa Ruby J, M.Pharm., Ph.D.

Asst. Professor

Department of Pharmaceutics

Acharya & BM Reddy College of Pharmacy

Soldevanahalli, Hesaraghatta Main Road,

Bengaluru-560107, Karnataka

ABSRTACT:

One of the most common parasitic infestations in the world, helminthiasis poses a serious risk to public health. A small number of anthelmintics have been primarily used to combat these nematodes. Finding innovative anthelmintics with a unique mode of action is imperative, as side effects and developing resistance to the already prescribed anthelmintic medications pose a serious threat. Hence, the current study was carried out with the aim to prepare and evaluate the Anthelmintic activity of an F1, F2 and F3 formulations for herbal syrup of kernel seed of *Mangifera indica.* Three different batches of this formulations were prepared and then screened for anthelmintic activity for each batch with different concentration such as 25 mg/ml, 50 mg/ml and 100 mg/ml along with standard drug Albendazole, whereas control group received only 2 % gum acacia. It was observed that, the standard drug Albendazole at all concentration (25 mg/ml, 50 mg/ml and 100mg/ml) exhibited highest anthelmintic activity

and F3 formulations shown the significant (P<0.001) activity with all different concentrations, whereas F2 formulation resulted as moderate and F1 formulation shown less significant activity.

Keywords: *Albendazole, Helminthiasis, Mangifera indica, Herbal syrup, Anthelmintic.*

1. Introduction:

A group of antiparasitic medications known as anthelmintics helps the body get rid of internal parasites like worms. It is used to treat helminthiasis, a disease caused by helminth infection. Animals with infections are also treated with these medications. Without seriously harming the host, anthelmintics drive out worms from the body by either stopping their growth or killing them. In the tropics, helminths are acknowledged as a significant threat to animal production. Both humans and animals are impacted by parasitic helminths, which cause significant suffering and restricted growth. Certain synthetic medications are available to treat these types of illnesses, but because of their high expense and side effects, research is being done to produce safer and more effective medications. Many synthetic substances have anthelmintic properties, including diethyl carbamazine, albendazole, mebendazole, thiabendazole, and derivatives of piperazine [1]. Any anthelmintic drug's action is to either kill or deaden the worm and remove it from the body. Among the drugs that are typically available are levamisole, piperazine, thiabendazole, mebendazole, and albendazole. The most often reported adverse effects of all these commonly used chemicals include headache, dizziness, nausea, vomiting, diarrhoea, and sleepiness. Nature was essential in healing diseases before the dawn of human civilization. The significance of traditional plants for medical purposes was discovered by ancient humans. Research on a wide range of herbs, including those with antidiabetic, anti-inflammatory, antifungal, antiseptic, and immune-boosting properties, is being conducted.

Mangifera indica (MI), also known as **mango, aam**, it is one of the important medicinal plant used in Ayurvedic and indigenous medical systems for over 4000 years [2] belong to genus *Mangifera* which consists of about 30 species. of tropical fruiting trees in the flowering plant family Anacardiaceae. According to ayurveda, varied medicinal properties are attributed to different parts of mango tree. Mango is known by various names around the world, for example, Manja in Arabic, Mannko in Greek, Am or Ambi in Hindi, Amba in Sinhala, Mangue in French, Mango in Finnish, Mango in Dutch, Mangue in German, and

Mampalam in Tamil [3]. Both ripe and unripe mango fruits are used as pickles, Jam, juice, by people as jam, pickles, juice, nectar, spicy powder, oil, cereal and sauce flakes [4]. The peel of mango fruits is said to be rich in fiber, vitamins C and A, amino acids and polyphenols [5]. It has also been noted that mango seeds are a great source of polyphenols [6]. Since from ancient times, Mango fruit is widely consumed as food, but since ancient times, different components of the tree have also been utilized medicinally, primarily in Southeast Asian and African nations [7]. A thorough assessment of phytochemicals, their biological effects, and the pharmacological and ethnomedicinal qualities of *M. indica* is lacking, despite the abundance of information regarding the plant's usage in pharmacology and ethnomedicine.

Taxonomy of *M. indica:*

The Anacardiaceae family includes the genus Mangifera. The most prevalent species in the genus Mangifera is *Mangifera indica*, which has over 69 distinct species [8,9]. The *M. indica* plant is an evergreen tree with a spreading canopy that can reach heights of 8 to 40 meters [10]. The bark of *M. indica* is superficially broken and has a thick, brown-grey colour. The leaves vary in size and range in length from 15 to 45 cm. The length of the leaf petiole varies from 1 to 10 cm [11].

Table 1: Taxonomical description of *M. indica*

Kingdom	**Plantae**
Class	Mangoliopsida
Phylum	Mangoliophyta
Order	Sapindales
Family	Anacardiaceae
Genus	Mangifera
Species	Indica

The fruits of ***M. indica*** (Figure 1) come in a variety of forms, including linear-oblong, ovate-lanceolate, oval, roundish-oblong, and oblong. Certain mango types include green, red, or yellow leaves; the upper surfaces of the leaves are often glossy. Male and hermaphrodite flowers are produced in the same panicle in *M. indica* blooms; their size varies in diameter almost 6-8mm, flowers 4000-5000 small flowers are red or purple spot petals [12,13].

***Figure 1: M. indica* fruits (a) and Kernel seeds (b) (c).**

Reported Phytochemicals in Different Parts of *M. indica*

Phytoconstituents of leaf reported on polyphenols and phenolic acids such as gallic acid, catechin, kainic acid, quercetin, hyperin, mangiferin, ellagic acid, amino acids such as glycine, alanine, lucein, valine, leucine, and γ-aminobutyric acid, terpens like α-pinene, β-pinene, δ-elemene, taraxerol, β-elemene, α–cubebene, camphene, γ–cadinene, lupeol, phytosterols like α, β, and γ-sitosterol [14-19]. Fresh fruits contains triterpenoids like α-amyrin, β-amyrin, methyl mangiferonate, methyl mangiferolate, quercetin 3-ara, quercetin 3-rha, isomangiferin gallate, mangiferin gallate, Carotenoids include β-carotene, zeaxanthin cis-violaxanthin, cis-neoxanthin, luteoxanthin, neochrome [20]. Root contains Sterols include β-sitostero and 3-methoxy-2-(4′-methyl benzoyl)-chrmone [14-19]. Bark part contains saponin triterpenoids includes indicoside A and B, phenolic acids like gallic acid, bezoic acid, catechin, mangiferin, protocatechuic acid [21, 22]. Seed and kernel parts shows the presence of fatty acids like stearic acid, eicosanic acid, oleic acid, linolenic acid, sterols like stigmosterol, campesterols, sitosterol, terpens like, limonene, α-pinene, β-pinene, myrcene [23]. Flowers contains amino acids, phenolic acid like gallic acid, mangiferin, ellagic acid and quercetin, terpenes like limonine nerol and α-pinene [24].

A thorough assessment of phytochemicals, their biological effects, and the pharmacological and ethnomedicinal qualities of *M. indica* is lacking, despite the abundance of information regarding the plant usage in pharmacology and ethnomedicine. As a result, we provide this review as a current and thorough analysis that focuses mostly on phytochemicals, some of their documented bioactivities, and the pharmacological and ethnomedicinal qualities of *M. indica.*

Hence the present study focused on the extraction, formulations and screening of anthelmintic activity of herbal syrup.

2. Methodology:

2.1. Plant material

Fruits of *Mangifera indica* were collected from local area Bangaluru, Kernal seeds was authenticated by **Dr. S. Mutheeswaran, Scientist, xavier Research Foundation, Palayan kottai, Tamil Nadu.** Voucher specimens (MI/2022-23/0204) are kept at the Acharya & BM Reddy College of Pharmacy, Soladevanahalli, Bengaluru. India.

2.2. Extraction of plant material

Around 1.5 kg of the dried kernel seed of *Mangifera indica* was taken and then coarsely powered and set aside for maceration with petroleum ether for 7 days in a closed vessel with occasional shaking. The liquid was strained off and the marc was kept for second maceration with 70 % of alcohol for 2 days. The marc was pressed to recover the occluded solution. Both the extracts were subjected to evaporation, resulted as reddish-brown extract and pale yellowish colour extract for was obtained. The yield was found to be 34 and 31 g respectively

2.3 Phytochemical Evaluation for kernel seed extracts:

The phytochemical screening [25] were carried out for the both petroleum ether and alcoholic extracts of kernel seed of *Mangifera indica* and the results were depicted [26] in the Table 2.

Table 2: Phytochemical screening of petroleum ether and alcoholic extracts of *M. indica*

Tests	Petroleum ether extract	Ethanolic extract
Alkaloids		
a. Mayer's test	-ve	-ve
b. Dragendrof's test	-ve	-ve
Steroids		
a. Salkowski test	+ve	+ve
b. Libererman Burchard test	+ve	+ve
Carbohydrates		
a. Molisch's test	-ve	+ve
b. Benidict's test	-ve	+ve
Flavonoids		
a. Foam tests (Saponins)	-ve	-ve

b. Shinoda test	-ve	-ve
Amino Acid and Proteins		
a. Ninhydrin test	-ve	-ve
Phenolic Compounds and Tannins		
a. Neutral $FeCl_3$ test	-ve	-ve
b. Dil. Iodine test	-ve	-ve
c. Dil. HNO_3 test	-ve	+ve
d. Dil NH_4OH test	-ve	+ve
e. Acetic acid test	-ve	+ve

Note: - '-'Negative '+' Positive

2.4 Formulation of herbal syrup:

Mangifera indica alcoholic seed extract was utilised for formulating the simple syrup. 20 ml of simple syrup was prepared by taking 4-5 ml of distilled water in beaker and 12g of sugar was added, i.e.,66.67 (w/v) as per IP. Simple syrup was prepared by heating to dissolve the sugar completely by continuous stirring. This results in a super saturated solution of sugar in water. Solution of extract was prepared in another beaker. This solution of extract was then added to sugar solution by continuous stirring in hot condition. The excipients were separately dissolved in water and then added to above solution. The volume of syrup was made up to 100 ml by adding required amount of distilled water and the *Mangifera indica* syrup was prepared. The formulation composition of the syrup is given in Table 3.

Table 3: Formulations (1-3)- Syrup from alcoholic seed extract of Mangifera indica

Sl. No	Ingredients	F1 Formulation (mg/ml)	F2 Formulation (mg/ml)	F3 Formulation (mg/ml)
1	*Mangifera indica* ethanolic Extract	40g	80g	120g
2	Methyl Paraben	8g	8g	8g
3	Propyl Paraben	0.8g	0.8g	0.8g
4	Citric acid	8g	8g	8g
5	Sodium benzoate	4g	4g	4g
6	Sucrose	24g	24g	24g
7	Distilled water	200ml	400ml	600ml

2.5 Evaluation of Herbal Syrup for all formulations (F1, F2 and F3 formulations) in table 4.

a. **Organoleptic properties:**

   The colour, taste and odour of the herbal syrup were determined after preparation.

b. **pH:**

   The pH of the formulated herbal syrup was determined by using pH Meter.

c. **Viscosity:**

   The viscosity of the syrup was determined by Brookfield's viscometer.

d. **Determination of crystal growth:**

   The crystal growth was determined after 24 hr of the preparation

2.6 Screening of Anthelmintic activity for different formulations of herbal syrups (F1, F2 and F3) and marketed drug Albendazole

Earth worms were procured from local suppliers of Bengaluru during the experiment was carried out. These worms were thoroughly washed with normal saline to remove the adhering material and sorted out for uniform size and length. These worms were kept in 6 % dextrose solutions for acclimatization [27]. The worms which showed the normal motility were selected for the activity. Thirteen Petri-dishes of equal size were selected and washed. Different herbal syrup (F1, F2 and F3 formulations) containing 25mg/ml, 50mg/ml and 100mg/ml were added to the Petri dish containing earthworms and the paralysis and death time were noted. The same procedure was repeated for the marketed Standard drug Albendazole. In each petri dish 3 earthworms. These earth worms were observed for their motility spontaneous motility and evoked responses. The paralytic time was recorded at different time intervals. Immediate after inhibition of response to external stimuli, the worms were placed in fresh water and observed for the recovery. Duration required for final recovery or death was noted. Mean paralytic score was plotted against time and by keeping the view of reported method and Albendazole was selected as reference standard.

The death time or total paralysis time were observed at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water (500ºC), which stimulated and induced movements if the worm was live. Two independent experiments were carried out for each observation to confirm the result. Worms were observed at regular intervals for evoked response, paralysis and death and the time of paralysis and death in all the three different concentration was recorded given in **table 4.**

Table 4: Time taken for evoked response, paralysis and death in each concentration for F1, F2 and F3 formulations.

Treatment Groups	No. of earth worms tests	Conc. (mg/ml)	Mean evoked response in min. ± SEM	Mean Paralysis response in min ± SEM	Mean death response in min ± SEM
Control	3	-	-	NP	ND
F1 Formulations	3	25	4.93±0.15	6.73±0.15	8.5±0.18
	3	50	4.32 ±0.17	5.33±0.15	7.98±0.20
	3	100	3.75±0.07	5.16±0.09	7.09±0.10
F2 Formulations	3	25	2.86 ±0.05	5.05±0.15	6.96±0.12
	3	50	2.95± 0.18	3.35±0.07	6.34±0.19
	3	100	2.14±0.11	2.33±0.11	4.14±0.15
F3 Formulations	3	25	2.93±0.15	4.73±0.15	4.85±0.15
	3	50	2.22 ± 0.11	3.33±0.15	3.59±0.20
	3	100	2.13±0.05	2.36±0.09	2.29±0.10
Albendazole (Std)	3	25	2.77 ± 0.14	3±0.12	4.09±0.09
	3	50	1.92 ±0.12	2.1±0.05	3.32±0.26
	3	100	1.55 ±0.42	2.16±0.01	1.73±0.21

3. RESULTS

3.1 Preliminary phytochemical screening:

Results of phytochemical screening for both petroleum ether and alcoholic extract of *M. Indica* was shown in **table 3**. The ethanolic extracts shows the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds and tannins.

Note: For petroleum ether extract, we did not proceed further for herbal formulation for syrup from petroleum ether extract, because it gave only positive test for steroids during phytochemical screening.

3.2 Evaluation parameters of Herbal Syrups:

Table 5: Evaluation parameters for all three Formulations (1-3) of herbal Syrup from alcoholic seed extract of *Mangifera indica*

Sl. No	Parameter	F1 Formulation	F2 Formulation	F3 Formulation
1	Colour	Pale Yellowish orange	Pale Yellowish	Pale Yellowish
2	Odour	Aromatic	Strongly aromatic	Pungent
3	Taste	Slightly Bitter	Bitter	Bitter
4	PH	6.8	6.9	7.2
5	Viscosity	3.60cp	3.65cp	3.66cp
6	Crystal growth	None	None	None

3.3 Anthelmintic activity for different formulation of herbal syrups:

Many traditionally used herbs have proved a potent anthelmintic activity by various experimental model. **Three formulations (F1, F2 and F3),** it was observed that the three different formulations of alcoholic extract of kernel seed of *Mangifera indica* herbal syrup shows different levels of on anthelmintic activity at concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml with reference to albendazole as a standard drug by using adult earth worm **Figure 2.**

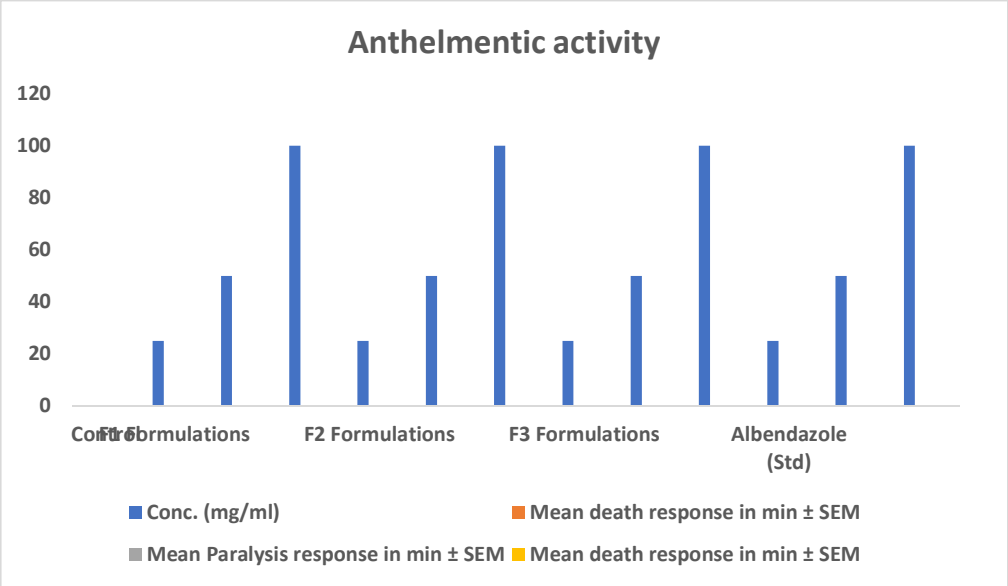

Figure 2: Anthelmintic activity of Herbal syrup (F1, F2, F3 Formulations)

F3 Formulations: Anthelmentic activity

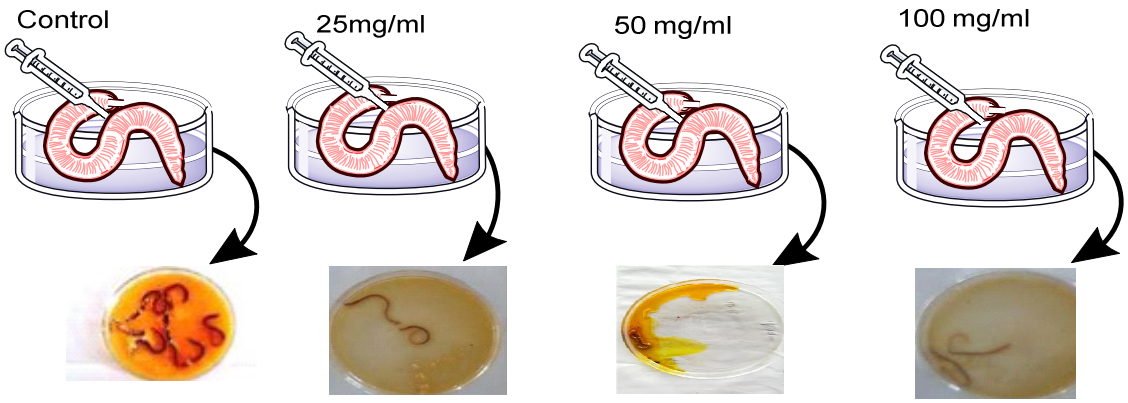

Figure 3: F3 Formulations

F2 Formulations: Anthelmentic activity

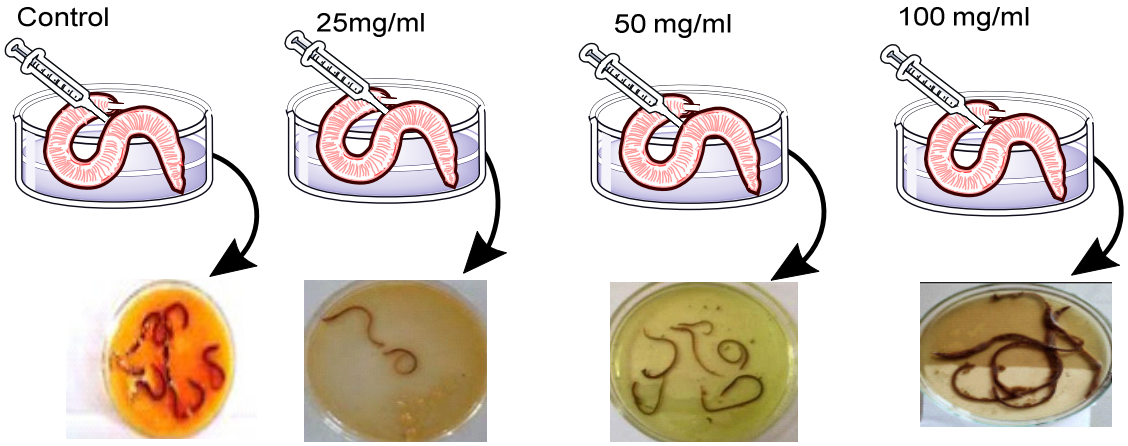

Figure 4: F2 Formulations

F1 Formulations: Anthelmentic activity

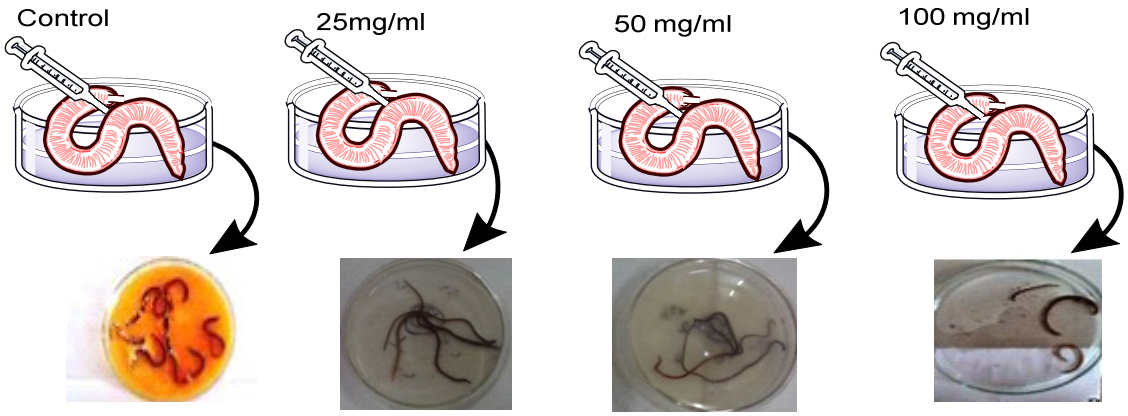

Figure 5: F1 Formulations

Figure 3: F3 Formulations: Anthelmintic activity of herbal syrup from kernel seed extract
Figure 4: F2 Formulations: Anthelmintic activity of herbal syrup from kernel seed extract
Figure 5: F1 Formulations: Anthelmintic activity of herbal syrup from kernel seed extract

4. DISCUSSION

The anthelmintic activity of final herbal syrup formulation was found to be more significant in F3 formulations and standard drug Albendazole [28] by decreasing time in evoke response, paralysis time and death time also, where F2 formulations shows moderate activity and F1 formulations exhibited less activity in all concentration. The result of anthelmintic activity showed due to the presence of secondary metabolites like tannins in the extract of *M. inidca* shown similar effects. Another possible mechanism for anthelmintic effect of tannins is that they can bind to free protein in the gastro intestinal tract of host animal or glycol protein on the parasite and cause death [29]. Further the extract can be tested on various other Helminthes to ascertain the anthelmintic activity on a broader scale which is our future plan of research work since the for the preparation of herbal formulation from the kernel seed extract of *Mangifera indica* has shown significant anthelmintic activity. The present study reveals that the seed of *Mangifera indica* shows potent anthelmintic activity as *Mangifera indica* is easily available in the local market and cheaper therefore this plant could be categorized under anthelmintic herbal drug [30]. This plant, especially Kernal part could further become a key ingredient of anthelmintic herbal formulations. Even though the initial results are encouraging, majorly focused to understand its mechanisms [31] and its effectiveness of different concentration of *M. indica (*kernel seed*)* herbal syrups in controlling parasitic worms.

5. CONCLUSION:

Kernel seeds of *Mangifera indica* have proved to be effective natural remedy against helminthiasis. The herbal syrup prepared from different formulations like F1, F2 and F3 herbal syrups, these three herbal formulations were found to be equal effective as that of standard Albendazole and also its shows synergistic effect. As these have exhibited potency in a very low concentration like F1 formulations, so they provide a safer, effective and easily available Anthelmintic remedy.

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