

TO EVALUATE ANTHELMINTIC ACTIVITY OF POLYHERBAL SYRUP ON EARTHWORM

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Abstract :

Helminthiasis is One of the most common diseases in the world, particularly in tropical nations, is the condition brought on by worm infestation. Anthelmintic is a drug that kills or removes gastrointestinal worms. "Dewormer" or "wormer" are the more popular names. Anthelmintics are also known as parasiticides, endectocides, nematocides, parasitic, and drenches. The current investigation was carried out with the aim to evaluate the anthelmintic activity of an herbal formulation. The Hydroalcoholic extracts of the leaves of Aegle Marmelos (Rutaceae), Murraya Koenigii (Rutaceae), flower buds of Eugenia caryophyllus (Myrtaceae), were screened for anthelmintic activity using the adult earthworm *Pheretima posthuma*. The herbs were coarsely powdered and extracted in hydroalcoholic solution. These extracts were first screened for their anthelmintic activity and then formulating herbal syrup which was again evaluated for its anthelmintic potential. Finally, Aegle marmelos and murraya koenigii leaves (200 mg and 400 mg) and clove (0.7 mg) were combined to make a herbal syrup, and 200 mg and 400mg of this combination paralysed earthworms. Substantial death occurs at 200mg at 4 min 09 sec in aegle marmelos and 3min 10 sec in murraya koenigii and 400 mg at 4 min 3 sec in aegle marmelos and 3 minutes, 4 sec. in murraya koenigii and 400 mg. Albendazole suspension was used as standard during both the screening and evaluation like density, specific gravity, pH, organoleptic characteristics.

Keywords : *Anthelmintic Activity , Polyherbal Formulation , Pheretima posthuman , Aegle Marmelos , Murraya Koenigii*

INTRODUCTION :

1.1 Helminthiasis :-

Helminthiasis is One of the most common diseases in the world, particularly in tropical nations, is the condition brought on by worm infestation.^[1]The most prevalent infectious agents for people in impoverished nations are helminths, which also cause a heavy burden of disease globally and increase the risk of pneumonia, eosinophilia, anaemia, and malnutrition.^[2] It is an infection of one or more intestinal parasitic worms, such as hookworms (*Necator americanus* and *Ancylostoma duodenale*) or whipworms (*Trichuris trichuria*) or roundworms (*Ascaris lumbricoides*). The symptoms of helminthiasis include abdominal pain, diarrhoea, fever, fatigue, enlarged liver, enlarged spleen, cough, eosinophilia, asymptomatic gastrointestinal inflammation, malnutrition, bowel obstruction, anaemia, dehydration, bloody diarrhoea, chest pain, vomiting, constipation, weight loss, distended abdomen, itchy skin, eye symptoms, malaise, headache, and itchy anus.^[3]

1.2 Anthelmintic :-

An anthelmintic is a drug that kills or removes gastrointestinal worms. "Dewormer" or "wormer" are the more popular names. Anthelmintics are also known as parasiticides, endectocides, nematocides, parasitics, and drenches. This comprises both flat worms, such as flukes and tapeworms, and round worms, such as nematodes.^[4] They may alternatively be referred to as vermicides (killing) or vermifuges (astonishing). This comprises both round and flat worms, such as nematodes and flukes, as well as tapeworms and other flat worms.^[5] According to reports, meals made from animals may include anthelmintic chemicals that are highly toxic to humans and pose a substantial risk to human health.^[6] Research on the anthelmintic activity of medicinal plants has a great impetus because herbal agents make a superior solution because they are nontoxic and economically viable.^[7]

1.3 *Murraya koenigii* :-

The rutacea family includes *Murraya koenigii*, sometimes known as the curry leaf tree, which may be found all over South East Asia and Sri Lanka. It is well known for having therapeutic qualities such as antibacterial, antifungal, cytotoxic, antidiarrheal, anti-inflammatory, and cytotoxicity.^[8] There are various ways to use *Murraya koenigii* as a useful plant, including extracts, essential oils, and direct use due to the presence of the active ingredients bismahanine, murrayanine, murrayafoline-A, bi-koeniquinone-A, bismurrayaquinone, mukoenine-A, mukoenine-B, and mukoenine-C. Other active ingredients include murrastif Quinone A and koenigine-Quinone B are used by common people for therapeutic purposes.^[9] The curry leaf tree is used by traditional healers to treat a variety of illnesses.^[10]

1.4 *Aegle Marmelos* :

Aegle marmelos L., also known as Bael in India and a member of the Rutaceae family, is an important fragrant medicinal plant with a long history of use against a variety of ailments.^[11] The *Aegle marmelos* tree, commonly

known as Shivaduma (The Tree of Shiva), is revered by Hindus and offered in prayers to the deities Lord Shiva and Parvati^[12] Biochemistry of *Aegle marmelos* such as Alkaloids, coumarins (marmelosin, marmesin, imperatorin, scopoletin), steroids, polysaccharides, phenylpropenoids, tannins, flavonoids, carotenoids, saponin, etc. have all been isolated from various tree parts, including leaves, fruits, wood, root, and bark. The leaves contain flavone, glycoside, Limonene, phenylethyl cinnamamides, aegelin, skimmianine (tannin), lupeol, rutin, marmesinin, -sitosterol, and others. Alkaloids such marmeline, ethylcinnamamide, halfordino, and shaidine are found in fresh leaves^[13]

1.5 Clove :-

Clove Mostly Uses In Ayurvedic Medicine. Lavang Is The Most Common Name For It. The Myrtaceae Family Includes The Expensive Spice Clove (*Syzygium Aromaticum*). The Primary Application Of Clove Is In Food Preparation. Due To Its Antibacterial, Antiviral, Anti-Inflammatory, Anti-Diabetes, And Antioxidant Effects, Clove Oil Is Employed^[14]. One of the most important sources of phenolic compounds is clove, which also contains alpha-humulene (0.55%), alpha-terpenyl acetate (0.1%), and methyl eugenol (0.2%). Other phenolic compounds found in clove include eugenol (80%–90%), eugenyl acetate (15%–17%), beta-caryophyllene (5%–12%), and eugenyl^[15].



Fig No.2 *Murraya koenigii*



Fig No.3 *Aegle marmelos*



Fig No.4 Clove

2. Material And Method :-

2.1 Collection And Authentication Of Plant

Dried Powder Of Leaves Of *Murraya koenigii*, *Aegle marmelos* And Dried Powder Of Bud Of Clove Collected From Sanchomee Herboveda Private Limited Traded As-Mankarnika Aushadhalaya 1015, Vedant Appt, Sadashiv Peth, Nagnath Par, Pune-411030.

2.2 Hydroalcoholic Extraction Of Plant Material

500 ml each of water (250 ml) and ethanol (250 ml) were used to soak 50g portions of the powdered leaves of *murraya koenigii*, *Aegle marmelos* and clove^[16]. a sterilised glass rod was used to stir the mixtures during the seven-day period and every 24 hours. Whatmann Filter Paper No. 1 was used to filter the extract to produce the

filtrate. The crude extract was obtained by keeping the filtrates in a water bath^[17]. The percentage yield was calculated by the formula = (extract obtained/powdered material packed) X 100^[18].

2.3 Drugs and Chemicals

Albendazole (200mg and 400mg) ,Ethanol ,Water, Normal saline, were use during experimental protocol. All chemicals are of the analytical and laboratory variety.

2.4 Analysis Of Hydroalcoholic Extract

2.4.1 PHYTOCHEMICAL SCREENING :

To identify the type of phytochemicals present in the extracts, a phytochemical analysis was conducted. Several of the tests described by Harborne and Odebiyi Sofowora were carried out for this reason^[19,20,21,22]

➤ Test for Alkaloids:-

✓ **Dragendorff's test:** - To the extract add Dragendorff's reagent, reddish brown precipitate indicates presence of alkaloids.

✓ **Mayer's test:** - To the extract add Mayer's reagent, cream colored precipitate indicates presence of alkaloids.

✓ **Wagner's test:** - To the extract add Wagner's reagent, reddish brown precipitate indicates presence of alkaloids.

✓ **Hager's test:** - To the extract add Hager's reagent, yellow precipitate indicates presence of alkaloids

➤ Test for Flavonoids:-

✓ **Alkaline reagent test:** - To the extract add few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicate presence of flavonoids

➤ Test for Phenolic compounds (Tannins):-

✓ **Ferric chloride test:** - Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

✓ **Gelatin test:** -To the extract add 1% gelatin solution containing 10% sodium chloride. Precipitate is formed

➤ Test for Proteins:-

✓ **Biuret test:** - To the extract (2ml) add Biuret reagent (2ml), violet colour indicates presence of proteins.

✓ **Hydrolysis test:** - Hydrolyse the extract with hydrochloric acid or sulphuric acid. Then carry out the Ninhydrine test for amino acids.

➤ **Test for Carbohydrates:-**

✓ **Molish's test:** -To the extract add few drops of alcoholic α -naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colour ring appears at the junction.

✓ **Fehling's test:** - Mix 1 ml Fehling's A and 1 ml Fehling's B solutions, boil for 1 min. add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow precipitate, then a brick red precipitate, are seen..

Test for Saponins glycosides: -

✓ **Froth formation test:** - In a test tube, add 2ml of a medication and water solution. After vigorously shaking the test tube, stable foam forms.

2.4.2 THIN LAYER CHROMATOGRAPHY (TLC) :

The Silica gel G slurry was used to prepare the TLC plates. The solvent system served as the mobile phase, and this served as the stationary phase. After the plates and solvent system were ready, the plates were allowed to air dry and the solvent system was allowed to reach saturation. The plates were activated in an oven at 105°C for 10-15 minutes after they had dried. Next, a capillary was used to apply a spot of syrup sample at a distance of roughly 1 cm above the bottom of the plates. After the sample spot dried, the plates were retained in the solvent system. About 75% of the stationary phase's time was spent with the solvent running. Then, two distinct areas of the drug's ingredients, murraya koenigii and aegle marmelos leaves were gathered and placed on a plate to be compared to the usual medication (caffeine and quercetin). The Rf of these spots was calculated by using the formula: $R_f = \frac{\text{Distance travelled by the Solute}}{\text{Distance travelled by the Solvent}}$ ^[23].

$$R_f = \frac{\text{Distance travelled by the Solute}}{\text{Distance travelled by the Solvent}}$$

2.4.3 UV SPECTROSCOPY ANALYSIS :

The extract was subjected to UV-visible spectrophotometric examination using a UV-visible spectrophotometer with a slit width of 2 nm and a 10-mm cell at room temperature. For proximate analysis, the extract was analysed using visible and UV light with a wavelength spanning from 300 to 800 nm. The extract was centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper for UV-VIS spectrophotometer examination. With the same solvent, the sample is diluted to a ratio of 1:10^[31]

2.4.4 FT-IR SPECTROSCOPY:

Fourier Transform Infrared Spectrophotometer (FTIR) determination:

FTIR analysis was used to examine the powdered dry leaves of *Murraya Koenigii* and *Aegle marmelos*. extracted using various solvents. In an FTIR spectrophotometer with a scan range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1} , 10 mg of the dried extract powder sample of each extract was loaded, and the FTIR spectrum was recorded. The presence of functional groups in the leaf extracts of *Murraya Koenigii* L. and *Aegle marmelos* was discovered using the FTIR spectra^[24].

3.FORMULATION OF SYRUP

Two simple syrup IP, or 66.67% (w/v), was created using combination of *Murraya Koenigii* with *E. caryophyllus* and another is *Aegle marmelos* with *E. caryophyllus* by following the instructions in IP. This two simple syrup was prepared in 10 millilitres. 6.667g of sugar was added to 1-2 ml of distilled water in a beaker. The liquid was heated while being continuously stirred to completely dissolve the sugar. This led to a highly saturated solution of sugar in water. The extracts were added to the solution in the amounts specified in table 1 once the sugar had completely dissolved. The extracts were added to the boiling sugar solution and thoroughly mixed to dissolve them. . After the extracts had completely dissolved, the syrup's content was increased to 10 ml by adding the necessary amount of distilled water. The syrup was created in the end. The Following Table contains the syrup's formulation makeup^[23]

Table No.1 :Composition Of Syrup

Sr.No.	Ingredient	Murraya Koenigii with E. caryophyllus		Aegle marmelos with E. caryophyllus	
		F1(200mg)	F2(400mg)	F1(200mg)	F2(400mg)
1	Sugar	6.667gm	6.667 gm	6.667gm	6.667gm
2	extract powder	200mg	400mg	200mg	400mg
3	Clove	0.0007gm	0.0007gm	0.0007gm	0.0007gm
4	Orange peel oil	0.1ml	0.1ml	0.1ml	0.1ml
5.	Distilled water	10ml	10ml	10ml	10ml

3.1.Herbal syrup standardisation:

The liquid herbal formulation, or syrup, was standardised based on the following principles.

1.Organoleptic Properties:

The syrup's flavour, colour, and aroma were assessed right away after it was made^[25].

2. pH: Using a pH metre, the syrup's pH was determined^[26].

3. Viscosity:

Brookfield's viscometer was used to measure the syrup's viscosity^[27]

4.Measurement of Crystal Growth:

After a 24-hour period, the crystal growth was measured^[28]

5.Density:

Density calculation procedure :

- 1) Use nitric or chromic acid to thoroughly clean the specific gravity bottle.
- 2) Rinse the bottle throughly with distilled water, ideally two or three times.
- 3) Rinse the bottle with acetone or another organic solvent if necessary, then dry.
- 4) Determine the mass of an empty, dry container with a capillary tube stopper (w1).
- 5) Place the cork on the bottle and fill it with an unknown liquid. Use tissue paper to dab away any extra liquid that may have collected outside the tube.
- 6) Place a bottle of an unknown liquid on an analytical balance, and weigh it (w2).
- 7) Determine the unknown liquid's weight in grammes (w3).

Density formula: : Density of liquid under test (syrup) = weight of liquid under test /volume of liquid under test = $w3/v$ ^[29]

6.Specific Gravity:

Process for calculating specific gravity

- 1) Use chromic or nitric acid to scrub the specific gravity bottle thoroughly.
- 2) Use filtered water to rinse the bottle at least twice or three times.
- 3) Dry the bottle after rinsing it with an organic solvent, such as acetone, if necessary.
- 4) Measure the weight of a dry, empty bottle using a capillary tube stopper.
- 5) Insert the stopper and fill the bottle with distilled water. Wipe the side tube of excess liquid with tissue paper (w2).

- 6) Place a water bottle with a stopper on an analytical balance (w2).
- 7) After drying and draining the liquid under test as described in steps 4 to 6, repeat the process by refilling the water.
- 8) On an analytical balance (w3), weigh a container with a cork and the liquid being tested.

Formula for specific gravity: Specific gravity of liquid under test (syrup) = weight of liquid under test /weight of water = w_5/w_4 ^[29]

4. Activity of Anthelmintic

4.1 Selection Of Earthworm

Indian adult earthworms (*Pheretima post-huma*) were used for anthelmintic activity. They were taken from moist soil and thoroughly cleaned with ordinary saline to remove all waste. Earthworms that were 3-5 cm long and 0.1-0.2 cm wide were used throughout the experimental period due to their physical and physiological resemblance to human intestinal roundworm parasites.



Fig no.5 *Pheretima posthuma*

Analysing the Anthelmintic Activity of Herbal Syrup :

Due to their morphological and physiological similarities to the intestinal round worm parasites in humans, adult earth worms (*Pheretima posthuma*) were gathered. To remove any adhering material, the earth worms were thoroughly washed with normal saline. Equal-sized petridishes were gathered, and the first one received 10 ml of just normal saline. The second and third petridishes received 10 ml of a 200 and 400 mg/ml albendazole solution, respectively. The hydroalcoholic extracts of *Murraya koenigii* Spreng, 10ml (200,400mg/ml) of the test solutions were then added to the fourth and fifth petridishes, respectively. Six earthworms of roughly comparable size were placed in each petridish, and the time it took for the paralysis (motion less) and complete death of the earthworms to be induced was recorded. The readings were verified by repeating the experiment ^[30].

RESULT AND DISCUSSION

EXTRACTIVE VALUE :

Table no.2 Extractive value

SR.NO	SOLVENT	EXTRACTIVE VALUE(%W/W)
1.	Hydroalcoholic extract of murraya koenigii	6.9
2.	Hydroalcoholic extract of Aegle Marmelos	26.72
3.	Hydroalcoholic extract of Clove	20

Fig. no. 6 Extraction Process

PHYTOCHEMICAL SCREENING :

Results of the phytochemical analysis of the powdered curry leaf extract are shown in Table. The examination of the powdered curry leaf extract using various solvents revealed the presence of several elements including alkaloids, carbohydrates, saponins, tannins, protein, glycosides, and flavonoids.

Phytochemical test :

Table No. 3 Phytochemical Test

Sr.no	Plant constituent	Name of test	Observation	
			Murraya Koenigii	Aegle Marmelos
1	Alkaloid	Mayer's test	+	+
		Hager's test	+	+
		Dragendorff's test	+	+
2.	Phenolic compounds (Tannins)	Ferric chloride test	+	+
		Gelatin test	+	+
3	Flavonoids	Alkaline reagent test	+	+
4	Proteins	Biuret test	-	-
		Hydrolysis test	-	-
5	Carbohydrates	Molish's test	+	+
		Fehling's test	+	+
6	Saponins glycosides	Froth formation test	+	+

THIN LAYER CHROMATOGRAPHY (TLC):

Table no.4 :TLC Result with RF value

Sr.no		Drug	Solvent system used	Detection reagent	RF value
1	Alkaloid	Curry leaves extract	Toluene:Ethyl acetate:Acetic acid [5:4:1]	Iodine chamber	0.57
		Caffeine (std)			0.67
		Aegle Marmelos (Bael)leaves extract	Chloroform:Methanol:Glacial Acetic Acid[8.3:1.7:1]	Iodine chamber	0.75
		Caffeine (std)			0.67
	Flavonoid	Curry leaves extract	Chloroform:Methanol: water [4:3:1]	Iodine chamber	0.77
		Quercetin(std)			0.78
		Aegle Marmelos leaves extract			0.6
		Quercetin(std)			0.50

UV SPECTROSCOPY :

Table No.5 UV Observation

Sr.no.	Concentration (µg/ml)	Absorbance	
		Murraya koenigii	Aegle marmelos
1	2 (µg/ml)	0.0083	0.0055
2	4 (µg/ml)	0.0151	0.0072
3	6 (µg/ml)	0.0275	0.0099
4	8 (µg/ml)	0.0340	0.0109
5	10 (µg/ml)	0.0410	0.0141

Graph of murraya koenigii and Aegle marmelos :-

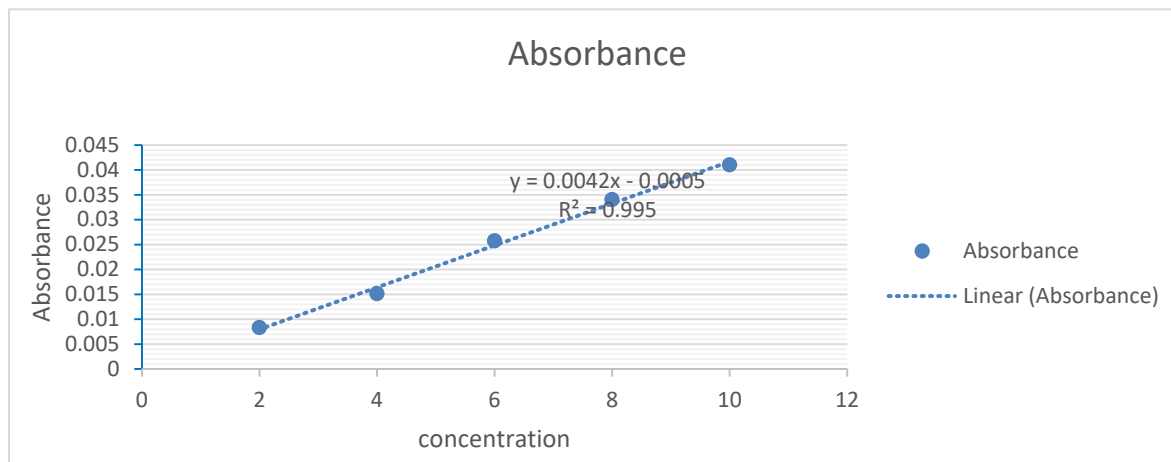


Fig .No.7. Calibration Curve Of Murraya Koenigii

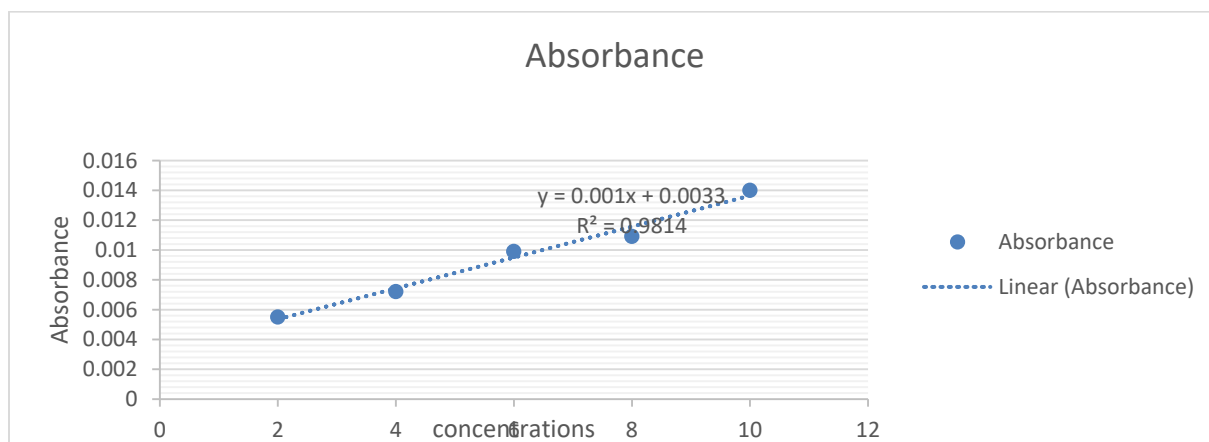


Fig no.8 Calibration Curve of Aegle Marmelos

Table no. 6- FTIR Observation

Sr. no.	Frequency cm^{-1}	Frequency cm^{-1}	Bond	Functional group
1.	3650-3600	3614.97	OH stretch	Alcohol
2.	3500-3100	3360.10	N-H stretch	Amine and Amide
3.	2900-2800	2819	C-H stretch	Aldehyde
4.	1680-1630	1642.13	C=O stretch	Amide
5.	1680-1600	1642	C=C stretch	Alkene
6.	1750-1730	1744.78	C=O stretch	Ester
7.	1300-1000	1144.05	C-O stretch	Ester

Aegle marmelos:-

IR INTERPRETATION OF HYDROALCOHOLIC EXTRACT OF AEGLE MARMELOS –

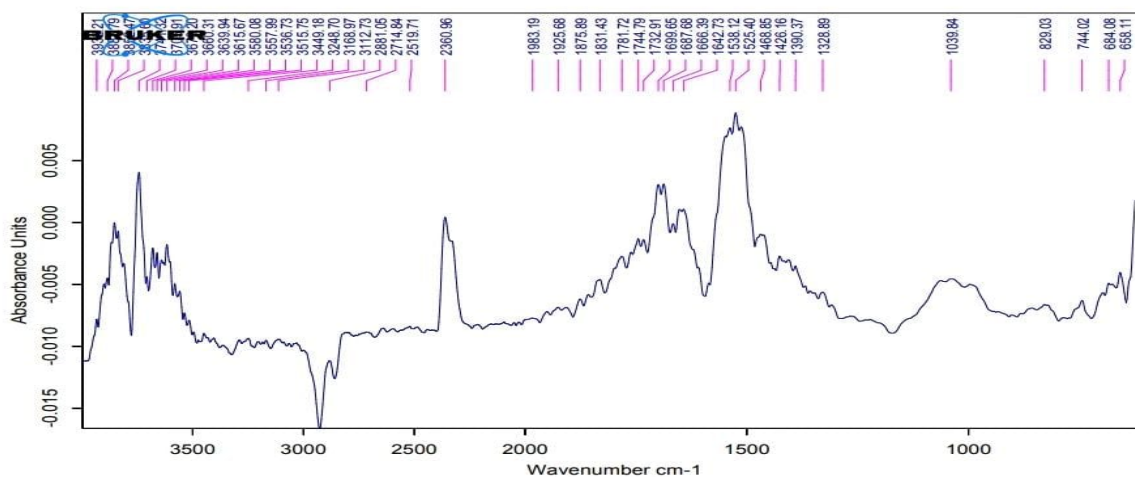


Fig no.11 FTIR Spectra of Aegle marmelos

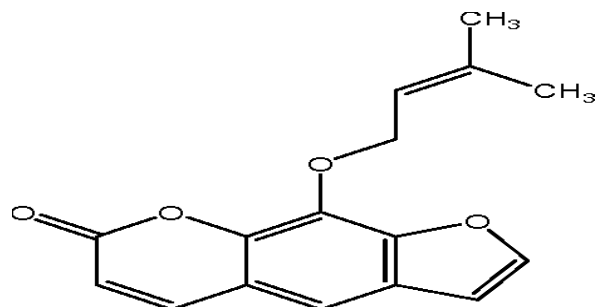


Fig no.12- Structure of Marmelosin

Table no. 7 FTIR Observation

Sr. no.	Frequency cm^{-1}	Bond	Functional group
1.	3355	OH stretch	Alcohol
2.	2922.5	C-H stretch	Alkane
3.	1732.2	C=O stretch	Aldehyde
4.	1642.1	C=C stretch	Alkene
5.	1613.1	C=C stretch	Ketone
6.	1460	C-O stretch	Carboxylic acid
7.	1255.4	C-O stretch	Esters

EVALUATION OF FORMULATION :

The physical parameter such as color and appearance were observed for herbal formulations.

Table No. 8 Evaluation Parameter Of Syrup

Sr no.	Procedure	Murraya Koenigii Syrup		Aegle Marmelos Syrup	
		F1 (200mg)	F2 (400mg)	F1(200mg)	F2(400mg)
1.	Organoleptic Property				
	a.Colour	Greenish	Dark greenish	Brownish Orange	Dark brown
	b.Odour	Sweet Aromatic	Sweet Aromatic	Sweet Aromatic	Sweet Aromatic
	c.Taste	Sweet	Sweet	Sweet	Sweet
	d.State	Liquid	Liquid	Liquid	Liquid
	e.Appearance	Clear	Clear	Clear	Clear
2.	pH Determination				
	a.pH Paper	Neutral	Neutral	Neutral	Neutral

	b. Digital pH Meter	6.73	6.77	6.57	7.00
3.	Viscosity				
4.	Determination Of Crystal Growth	None	None	None	None
5.	TLC				
6.	Density	1.33 gm	1.34 gm	1.33gm	1.34 gm
7.	Specific Gravity	0.591 mg	0.592 mg	0.591 mg	0.592 mg

Activity of Anthelmintic:-

Table no.9 Observation of the Anthelmintic Activity performed with the Herbal Formulation

Sr.no	Treatment	Concentration (mg/ml)	Paralysis Time (min/sec)	Death Time (min/sec)
1.	Control		No Paralysis	No Death
2.	Albendazole Suspension (Standard)	200 mg	1 min 25 sec	4 min 36 sec
		400 mg	1 min	3 min 59 sec
3.	Curry Leaves Syrup	200 mg	1 min 52 sec	3 min 10 sec
		400 mg	51 sec	3 min 4 sec
4.	Aegle Marmelos (Bael)Syrup	200 mg	2 min 06 sec	4 min 09 sec
		400 mg	1 min 15 sec	4 min 3 sec

GRAPH OF ACTIVITY of murraya koenigii and aegle marmelos

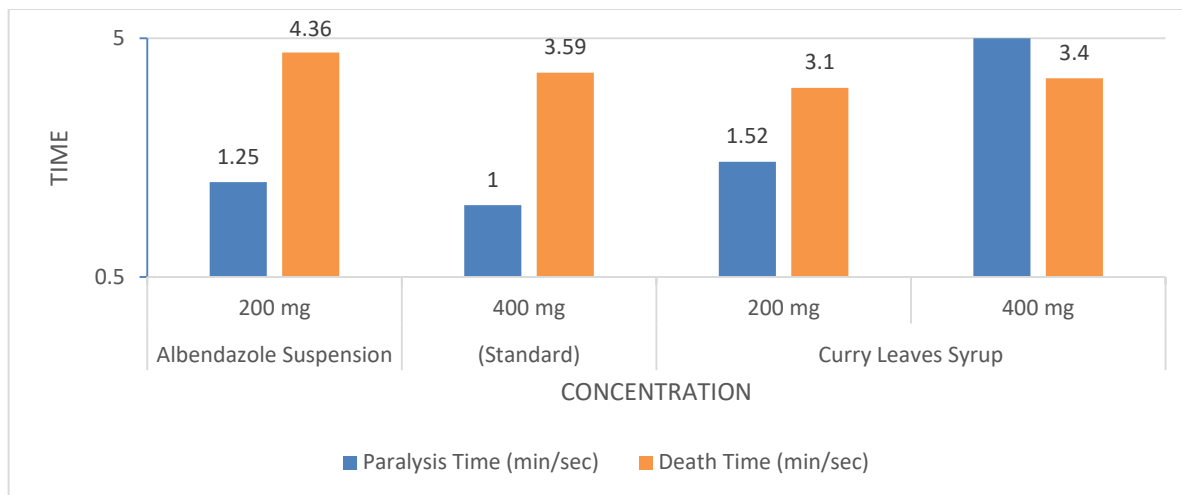


Fig.No.13 Graphical Representation Of Anthelmintic Activity Of Murraya Koenigii

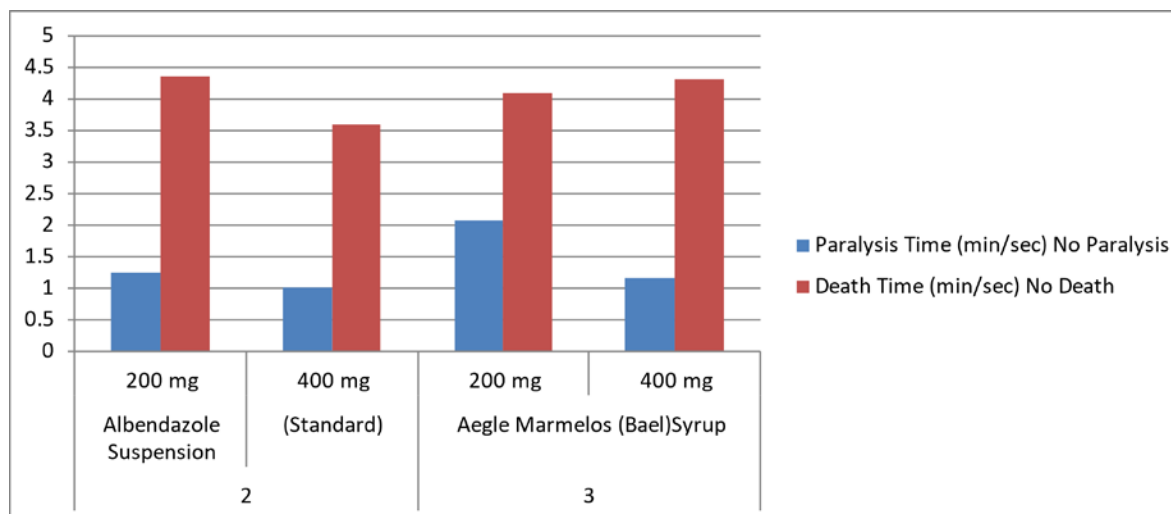


Fig. No. 14. Graphical Representation Of Anthelmintic Activity Of Aegle Marmelos

OBSERVATION :



Fig.no-14.Control test (normal saline)



Fig.No-15. Paralysis State

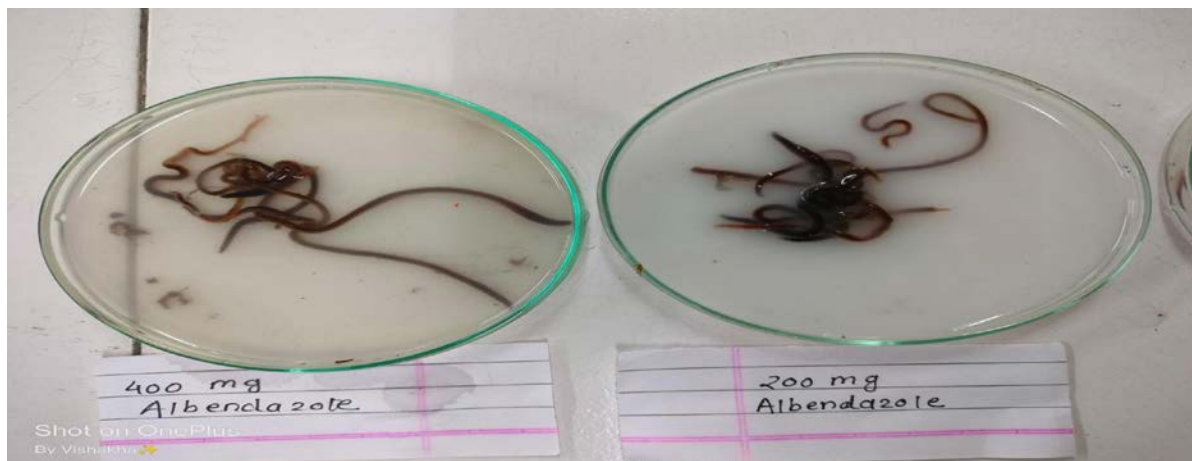


Fig.no-16 standard drug(Albendazole 200mg suspension and Albendazole 400mg suspension)



Fig.no-17.curry leaves syrup 200mg



Fig.no-18.curry leaves syrup 400mg



Fig.no. 19 A.Marmelos leaves syrup 200mg



Fig.no.20 A.Marmelos leaves syrup 400mg

Conclusion:

It can be concluded that active constituents responsible for anthelmintic activity are present in the herbal syrup of leaves murraya koenigii. And aegale marmelos .murraya koenigii with clove and Aegal marmelos with clove of Hydro alcoholic extracts were made into an oral syrup that complied with the requirements of the Indian Pharmacopoeia 1966, Second Edition. These two herbal medicines have a variety of therapeutically effective components that are combined for improved therapeutic results in the formulation of the polyherbal syrup. Due to the presence of alkaloid and flavonoid, which is a significant inhibitor of drug metabolism, the combination of murraya koenigii extract with clove extract and another Aegal marmelos extract with clove extract in the herbal syrup leads in the augmentation of bioavailability. This innovative polyherbal syrup may function as a health tonic and offer a number of therapeutic advantages when used as a disease preventative. To meet the required standards, these formulations need to be further standardised for expedited stability studies.

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