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RESEARCH ARTICLE

Formulating, Pharmacognostic and Physicochemical Insights of Polyherbal Vermifuge Formulation

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ABSTRACT:

The objective of present investigation was to prepare and standardize the churna from the plant materials i.e. Ginger, Garlic, Clove, Neem for anthelmintic activity. The Churna was prepared by taking plant materials, grinding it and was sieved from 80mesh size. The prepared fine powder was then used for testing and standardization. The physicochemical, phytochemical, flow property, TLC was determined. The in-vitro study was carried out on the earthworms for the effect of prepared churna to show the anthelmintic activity. Also the stability study was carried out. The raw material was prepared and authenticated. The churna was prepared by mixing all the ingredients in the fine form. The aqueous and methanol extract of prepared churna showed anthelmintic activity in dose – at 10, 20, and 40 mg/ml concentration for worms. It can be concluded that the active constituents responsible for anthelmintic activity present in the aqueous and methanol extract of prepared churna. The prepared churna is stable, safe, and effective against the helminths and can we used as an anthelmintic.

KEYWORDS: Churna, Anthelmintics, Helminthes, Vermifuge, In-vitro study.

INTRODUCTION:

The Helminthes infections are among the most widespread infections in humans, disturbing a huge population of the world. To evaluate compounds with anthelmintic activity, a number of substances were analyzed using different species of worms, for example, earthworms, Ascaris, Nippostrongylus and Heterakis. From all these species, earthworms have been used widely for the preliminary evaluation of anthelmintic compounds invitro because they are similar to intestinal "worms" in their reaction to anthelmintics and are easily accessible.

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It has been verified that all anthelmintics which are toxic to earthworms are good to study as an anthelmintic. Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed are of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the muco-polysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This is due to irritation leading in paralysis. Commonly used anthelmintic drugs like piperazine citrate and albendazole by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. The indiscriminate use of

anthelmintics has led to the development of drug resistance worms. The problem of anthelmintic resistance, toxicity and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant developed drugs, in the form of extracts containing mixture of different plant secondary compounds. The utilization of plants for the treatment of diseases of human and animal origin continues to rise although with few studies demonstrating proof of these effects. Some plant extracts that were found to exhibit invitro anthelmintic activity are *Zingiber officinale*, *Azadirachta indica*, *Allium sativum, Syzigium aromaticum*. Aqueous and methanol extract of prepared churna showed anthelmintic activity in dose – at 10, 20, and 40 mg/ml concentration for worms. It can be concluded that the active constituents responsible for anthelmintic activity present in the aqueous and methanol extract of prepared churna.[1-3]

Collection and preparation of plant material:[2,5]

1. **Ginger:** The rhizome of *Zingiber officinale* was collected then dried in shade, powdered and passed through sieve no. 80 and lastly packed in a well closed container to protect them from moisture.
2. **Neem:** The plant material i.e. leaf of the *Azadirachta indica* was dried in shade, grinded, passed through sieve no. 80 and stored in air tight container and used for further standardization.
3. **Garlic:** The dried bulb of *Allium sativum* Linn was collected then dried in shade, powdered and passed through sieve no. 80 and lastly packed in a well closed container to protect them from moisture.
4. **Clove:** The dried flower bud of *Syzygium aromaticum* (Linn.) was collected then dried in shade, powdered and passed through sieve no. 80 and lastly packed in a well closed container to protect them from moisture.

Pharmacognostic and Physicochemical Investigation of Raw Materials and formulated Churna: [3-5]

1. Foreign matter analysis: Here the each drug samples are sieved and then with the help of magnifying lens checked for the presence of impurities. Then impurities and fines are considered as foreign matter and weighed and % foreign matter calculated.
2. Macroscopic study was carried out by color, odour, and taste for samples used in preparation of churna.
3. Microscopic examination method:

1. Ginger:

a. **Lignified fibres:** In powder add small amount of Phloroglucinol and stand for 2 min. Then add conc. HCl. and the pink colour fibres are stained.
b. **Starch grains:** In powder add small amount of iodine solution. Blue colour grains are observed.
c. **Parenchyma cells:** In powder add small amount of Phloroglucinol and stand for 2 min. Then add conc. HCl. and the pink colour fibres are stained.

2. Clove:

a. **Calcium oxalate crystals:** The powder was stained by Dil. Acetic acid. The insoluble matter was found to be calcium oxalate crystals.
b. **Spindle shape fibres:** In powder conc. H_2SO_4 was added. Needle shaped fibres was observed after 10mins.

3. Neem:

a. **Stone cell:** The Powder was stained by Conc. H2SO4.
b. **Calcium oxalate crystals:** The powder was stained by Dil. Acetic acid. The insoluble matter was found to be calcium oxalate crystals.

4. Garlic:

a. **Stone cell:** The Powder was stained by Conc.H_2SO_4
b. **Prismatic Calcium oxalate crystals:** The powder was stained by Dil. Acetic acid. The insoluble matter was found to be calcium oxalate crystals.
c. **Parenchyma cells:** In powder add small amount of Phloroglucinol and stand for 2 min. Then add conc. HCl and the pink colour fibres are stained.

5. Loss on drying:

To estimate loss on drying, the 5gm of dried powder were accurately weighed in a dried and tarred flat weighing bottle. The sample was dried to constant mass in a hot air oven at temperature 105ºC. The loss on drying was determined with respect to air dried powder.

6. Determination of ash value:

a. **Total ash:** The 5g of accurately weighed powder drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^0C until it's free from carbon and constant weight, cooled and weighed.
b. **Acid-insoluble ash:** The 0.10gm of Total ash obtained was boiled for five minutes with 25 ml of dilute Hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited, cooled and weighed. The percentage of acid insoluble ash was calculated with reference to powder. The test was carried all for all the ingredients.
c. **Water-soluble ash:** The 0.10gm Total ash obtained was boiled for five minutes with 25ml of distilled water, cooled and collect the insoluble matter on an ash-less filter paper, washed with hotwater and ignited for 15 minutes at temperature not exceeding 450^0C. The percentage of water-soluble ash was

calculated with the reference to powder. The test was carried all for all the ingredients.

7. Determination of extractable matter:

This method determines the amount of active constituents extracted with solvents from a given amount of herbal material. It is employed for materials for which as yet no suitable chemical or biological assay exists. Extraction is carried out by cold maceration method.

Cold Maceration: Place about 2.5gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Macerate with 10 ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allow to stand for 48 hours. Filter rapidly, taking care not to lose any solvent, transfer 25 ml of the filtrate to a tarred flat-bottomed dish and evaporate to dryness on a water-bath. Dry at 105 °C for 6 hours, cool for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per g of air-dried material.

8. Phytochemical investigation:

All drugs were extracted in methanol and extract was concentrated. These extracts were then subjected to various qualitative tests for identification of various plant constituents like alkaloid, glycoside, volatile oil, flavonoids, tannins etc.

Preparation of extract:

The dried powder material of ginger, clove, garlic and neem to about 50 gm, were thoroughly mixed, taken in 1 lit. Beaker and distill water in sufficient quantity was added, then it was kept for maceration for 48 hours. The aqueous and ethanolic extract obtained was filtered and concentrated on hot plate.[6]

Determination of anthelmintic activity:

1. **Preparation of reference standard drug and test formulations:**
   Piperazine citrate is taken as standard drug and the concentration of the standard drug was dissolved in 100ml of normal saline solution to get 1, 2, and 4ml of solution. Normal saline alone was used as control.

2. **Preparation of ethanolic extract:**
   The all ingredients are macerated using water and ethanol. The 10mg of the dried form of extract is been used for the study. The all ingredients are taken 10mg/ml in normal saline. And the extract of churna is taken in the 10, 20, 40mg/ml was taken for the study.

3. **Experimental worms:**
   All the 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 fecal matters.

Figure 1: Image of Pheretima Posthuma

4. **Experimental design:**
   The anthelmintic activity was performed according to the Ghosh et al., method. On adult Indian earth worm Pheretima posthuma as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Pheretima posthuma was placed in petridish containing three different concentrations (10, 20 and 40mg) of ethanolic extract of leaves of E. variegata. Each petridish was placed with 1 worm and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the death time of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when sensitive to external stimuli. The test results were compared with reference compound piperazine citrate (5, 10 and 15mg/ml) treated samples.[7, 8]

RESULT AND DISCUSSION:

1. Pharmacognostic and Physicochemical Investigation of Raw Materials:

1. Foreign matter analysis:

The foreign matter in the raw materials was found within the limit. And the formulated churna showed 1.08% of foreign matter. And thus it concludes the standard value for formulated churna.

Table No. 1: Foreign matter analysis

Sr. No.	Drug Materials	Foreign matter (%)	Inference
1	Ginger	0.76 ± 0.0529	Within limit
2	Clove	1.2167 ± 0.196	Within limit
3	Neem	1.12 ± 0.1582	Within limit
4	Garlic	1.3 ± 0.1312	Within limit
5	Formulated Churna	1.08 ± 0.141	Within limit

Loss on drying:

Loss on drying of (laboratory samples) and its ingredients are mentioned in Table No. 4. All ingredients of churna have shown loss on drying within the Pharmacopoeial limit. This indicates that these samples contain moisture content within the acceptable range.

Table No. 2: Results of LOD of ingredients and churna

Sr. No.	Plant material	Loss on drying* (%)
1.	Ginger	6.50± 0.0208
2.	Clove	4.30 ± 0.0252
3.	Neem	6.33 ± 0.0252
4.	Garlic	9.33 ± 0.231
5.	Prepared Churna	4.33 ± 0.0423

Determination of ash value:

The 5gm of churna sample was taken in the silica crucible. The silica crucible weight was noted (18.230gm) and the value was obtained for total ash value, acid insoluble ash and water soluble ash. The all the ash value of the plant materials was found with the limit. The formulated churna was also investigated for its total ash value, acid insoluble and water soluble ash values. And thus can be used for the standardization.

Determination of extractive value:

The extractive values of the ginger, clove, neem, and garlic was obtained. It was found that the water extractive values were more than that of alcohol. The formulated churna also determined for its extractive value and the water extract was more concentrated than that of alcohol.

Phytochemical investigation:

The presence of phytochemicals was investigated for all the raw materials and formulated churna. The presence of the alkaloid (mostly steroidal) and glycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with their antioxidant effect which is capable of reducing the nitrate generation which can interfere in local homeostasis that is essential for the development of helminthes. Monomers of condensed tannins are reported to have the capacity to bind the protein and prevent the growth of nematode third stage larvae. Another possible anthelmintic effect of tannins is binding to free proteins in the gastrointestinal tract of host animals or glycoproteins on the cuticle of the parasite resulting in the death of the parasite

Table No. 3: Results of ash value

Plant Material	Parameteres	Obtained Values (%)
Ginger	Total Ash Value	6.53 ± 2.722
	Acid Insoluble Ash	7.66 ± 0.577
	Water Soluble Ash	12.33 ± 1.527
Clove	Total Ash Value	4.8 ± 2.253
	Acid Insoluble Ash	10.66 ± 3.785
	Water Soluble Ash	11.667 ± 0.451
Neem	Total Ash Value	9.066 ± 3.477
	Acid Insoluble Ash	3.5 ± 1.5
	Water Soluble Ash	15 ± 3
Garlic	Total Ash Value	7.066 ± 2.610
	Acid Insoluble Ash	4.33 ± 1.04
	Water Soluble Ash	13.33 ± 5.859
Formulated Churna	Total Ash Value	20.26 ± 1.724
	Acid Insoluble Ash	7.6 ± 1.058
	Water Soluble Ash	12.66 ± 1.527

Table no.4: Extractive value of ingredients and Churna

Sr.No	Plant Material	Water Extractive Value		Alcohol Extractive Value	
		%	STD Limit	%	STD Limit
1.	Ginger	15.44 ± 0.846	NLT 10	4.38 ± 0.482	NLT 3
2.	Clove	13.84 ± 0.860	NLT 9	6.03 ± 0.947	NLT 3
3.	Neem	11.73 ± 0.266	NLT 7	8.29 ± 0.333	NLT 6
4.	Garlic	13.6 ± 0.952	NLT 9	3.92 ± 0.501	NLT 2.5
5.	Prepared Churna	14.93 ± 0.582		7.22± 0.241	

*NLT = NOT LESS THAN

Table No. 5: Results of Phytochemical investigation

Sr. No.	Test	Ginger (*Zingiber officinale*)	Clove (*Syzigium aromaticum*)	Neem (*Azadirachta indica*)	Garlic (*Allium sativum*)	Formulated Churna
1.	Alkaloids	+	+	+	+	+
2.	Glycosides	+	+	+	-	+
3.	Volatile oil	-	-	-	-	-
4.	Tannins	+	+	+	+	+
5.	Phenolic	-	+	-	-	-
6.	Saponin	+	+	+	+	+

Powder flow properties:

For checking the flow properties such as bulk density, tape density, Carr's index, angle of repose, Hausner's ratio were performed for laboratory formulated churna.

Table No. 6: Results of powder flow properties

Sr. No.	Powder flow property	Result obtained
1.	Bulk density (gm/ml)	0.474 ± 0.0019
2.	Tap density (gm/ml)	0.544 ± 0.0023
3.	Hausner's ration	1.147 ± 0.0097
4.	Carr's Index (%)	12.86 ± 0.7408
5.	Angle of repose	26.24

The result of powder flow property for formulated churna was determined. The cars index shows that the powder flow property is good. The angle of repose for the churna shows good type of flow property. Thus the overall flow property of the formulated churna was found to have good flow.

Anthelmintic activity:

Table No. 9: Anthelmintic activity of Aqueous and ethanolic (1:1) extracts of formulated churna and Piperazine citrate.

Sr. No.	Treatment	Concentration (mg/ml)	Paralysis Time (min)	Death Time (min)
1	Normal saline	0.9% NaCl	No paralysis	No Death
2	Piperazine citrate	05	26.30	39.20
3	Piperazine citrate	10	18.10	25.25
4	Piperazine citrate	15	10.40	16.30
5	Ginger	10	20.30	34.40
6	Clove	10	14.20	28.10
7	Neem	10	28.40	58.44
8	Garlic	10	19.05	29.30
9	Formulated churna	10	25.20	41.20
10	Formulated churna	20	19.40	34.50
11	Formulated churna	40	14.10	26.35

CONCLUSION:

Standardization of formulation is required to have assurance about biological activity. The efforts are made here to standardize churna in terms of various pharmacognostic, physicochemical and phytochemical parameters to establish reliable values for respective and correlate with further work on analytical study. It is concluded from the pharmacognostic, physicochemical and phytochemical studies that all the raw materials are genuine.

The batches of formulated Polyherbal Churna were evaluated for pH, foreign matter, macroscopic and microscopy.

The stability studied results within the limit and it assures the long term stability. The pharmacognostic, physicochemical and phytochemical parameters can now be laid down for this formulation. This formulation showed very good anthelmintic activity. Thus this research work we concluded that the developed new polyherbal formulation is suitable for anthelmintic activity.

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