

Anti Helminthic Activity and Phytochemical Screening of Ethanolic Extract of Brassica Nigra

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ABSTRACT:

BRASSICA NIGRA an important medicinal plant is one of the most widely cultivated species of the family **Brassicaceae**. It is highly valued from time immemorial because of its vast medicinal properties.

The present study provides all necessary information regarding its phytochemical investigations, pharmacological actions and medicinal properties like treatment of common cold, painful joints, and muscles and arthritis. Black mustard seed is used for causing vomiting, relieving water retentions by increasing urine production, and increasing appetite.

It gives an account of all the data and reports which have been appeared to prove its medicinal and nutritional importance. Its utility as a food product has also been extensively described. Every part of Mustard Seeds is said to have beneficial properties that can serve humanity so the whole plant can be extensive studied for further research aspects.

Brassica Nigra is the most significant genus in the family **Brassicaceae**, which is commonly known as mustard seeds in India. This study evaluates anthelmintic activity of ethanol seed extract of mustard seeds on India adult earthworms, **Pheretima posthuma** [annelid]. **Brassica Nigra** seeds were extracted by using soxhlet apparatus using Ethanol as solvent. Photochemical screening of ethanolic extract showed the presence of tannins, phenolics, flavonoids, terpenoids, steroids and proteins. Various concentration of [25,50,100mg/ml] of seed extract were tested for anthelmintic activity which involved the determination of the time of paralysis and time death of worms when compared to standard drug Albendazole. **Brassica Nigra ethanolic extract showed dose dependent increase in anti**

helminthic activity when compared to Albendazole.

KEY WORDS: - Brassica Nigra, Mustard seeds, Pheretima Posthuma

I. CHAPTER-1

1.0 INTRODUCTION:



FIGNO.1BRASSICA NIGRA SEEDS

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. 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. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anemia, eosinophilia and pneumonia and its

worldwide prevalence lies between 500 million to one billion annually approximately. Anti-helminthic drugs target the helminth parasitic worms and expel them from the body, either by stunning or by killing them. Ideally an anti-helminthic 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 anti-helminthes 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 anti-helminthics has limited the effective control of the parasites. This leads to renewed interest in screening of medicinal plants for their anti-helminthic activity. The traditional medicines hold a great promise as source of easily available effective anti-helminthic agents to the people, particularly in tropical developing countries. The present study was designed to investigate the anti-helminthic activity of flowers of plant *Brassica Nigra* family Brassicaceae. *Brassica* is an important genus in the family of Brassicaceae. The genus *Brassica* consists of 37 species of flowering plants in the Brassicaceae, many of which are important agricultural crops. Most are the herbaceous plants, while some are shrubs and very few vines. Brassicaceae have a bisymmetrical corolla, septum dividing the fruit, lack stipules and have simple leaves. Brassicaceae vegetables are highly diversified in Mediterranean Europe, Asia, North America. *Brassica nigra* is an annual plant cultivated for its black or dark brown seeds which are commonly used as spice. In Telugu it is called as *avalu*, in English mustard seeds in Tamil *kadugu*. It belongs to the family Brassicaceae. Mustard seeds are used for the common cold, joint pains, arthritis and other conditions. It has been used since ancient times to eliminate cavity effusion, relieve joint pains and numbness, alleviate cough, and reduce swelling. The ethanolic extract of crude drug of different concentrations were tested which involve determination of paralysis and death time. Albendazole is used as standard and it was found that the ethanolic extract of mustard seeds showed a better Anti-Helminthic activity. The aim of the present study was to investigate the Anti-Helminthic activity of mustard seeds (*Brassica Nigra*) using adult Earthworms.

1.1 HELMINTHIC DISEASES:

Diseases caused by helminthes they include:

1. Soil transmitted helminthiasis.
2. Round worm infections such as lymphatic Filariasis, dracunculiasis and onchocerciasis
3. Trematode infections, such as schistosomiasis, food-borne trematodiasis, including Fascioliasis, Clonorchiasis, Opisthorchiasis and paragonimiasis.
4. Ascariasis, Trichuriasis, Hookworm, followed by Schistosomiasis
5. Pinworm infection, Trichinosis, Whip worm disease.

1.2. Plant characteristics, origin and application of mustard:

1.2.1. The origin and distribution of mustard:

Mustard has been cultivated in many parts of Eurasia for centuries and is suitable for subtropical and temperate climate cultivars. Its main origin is considered to be Central Asia (north western India), followed by eastern India, central and western China, Myanmar, and Iran and its Near East. China is theorized to be the original region where varieties differentiated, with Sichuan Province possessing the highest degree of differentiation. The plant appears in some form in the diets of Japan, Africa, India, Bangladesh, China, Korea, Italy, Nepal, Pakistan, and African Americans. Common types in the United States include brown mustard (*Brassica bresseriana*) and Abyssinia mustard (*Brassica carinata*) (Cools & Terry, 2018; Miceli et al., 2014; Rakariyatham & Sakorn, 2002).

1.2.2. Mustard types:

There are many reports on the classification of mustard, but most of these classification studies adhere to the traditional morphological descriptions, and there is little agreement on morphological classification and variety classification.

In China, mustard is classified into four categories: root mustard, tuber mustard, leaf mustard, and stalk mustard, with 16 varieties. In other countries, mustard varieties are divided into the following four subgroups: *integrifolia*, *tsatsai*, *juncea* and *napiformis* (Cools & Terry, 2018; Meng et al., 2007).

Mustard plants can reach 150 cm in height. The young stems and leaves are bristly, the stems are erect, the lower leaves are smaller, the edges are notched or toothed, the upper leaves are narrow-

lanceolate, and the edges are not sparsely toothed or entire. Mustard usually blooms from March to May and bears fruit from May to June. Mustard has a short growing period, about 30 days from sowing to harvest. The suitable growth temperature is 15–22°C (Cools & Terry,2018; Meng et al.,2007; Rakariyatham & Sakorn,2002)**1.3. Research progress on chemical constituents in mustard:**

1.3.1 General nutrients:

Mustard leaves are rich in chlorophyll, β -carotene, ascorbic acid, potassium, calcium and other minerals (S.H. Lim et al.,2000). Mustard seeds contain protein, carbohydrates, dietary fiber and fat. In addition, they contain vitamins(such as vitamin C and K) and various trace minerals (such as Ca, Fe, Zn, Se, Cu, Mn, and Mg) and electrolytes (Na and K), etc (Campbell et al.,2012; Jaiswal et al.,2012). As stated above the specific nutrients and their content in mustard vary with the variety, location, growing area, and processing method. The nutrient content in Mustards of varying conditions were summarized in Table 1. There is insignificant difference in nutrient content between upper leaves and lower leaves of Mustard (Farnham et al.,2012). The macro nutrient content of protein, carbohydrates and fat in the seeds are significantly higher than the rest of the plant, while the dietary fiber is lower than that of rest plant. (Jaiswal et al.,2012). Meanwhile, Indian mustard (*Brassica juncea* L.) is an oil-bearing mustard whose seeds are rich in protein and oil (37–49%), and its prospects are promising (Beenish & Lal,2019; Fadhil et al.,2020).



FIGURE NO: 2Mustard oil

1.3.2 Health Benefits of Mustard Oil:

The health benefits of mustard oil are plentiful. It can improve your circulation and also works as a strong stimulant, making your entire body function well and stay healthy. Mustard Oil can aid various systems in your body such as the digestive system, respiratory system, and even your immune system to an extent.

Mustard oil used for cooking, it fights against bacterial and fungal infections and can be used on internal and external infections. It is extremely good for your muscles and their growth and development. Mustard Oil helps in the pain and inflammation caused by arthritis. Mustard essential oil is also well known for being an excellent toner and can also help relieve random problems like headaches, cough, cold, congestion, irregular bowel movements, and so on.

What is Mustard Oil?

Mustard oil is extracted from the mustard seed through steam distillation. This essential oil has varying connotations depending on where you come from in the world. For instance, in Asian countries, especially India and Bangladesh, Mustard Oil is widely used for culinary purposes and is fairly popular.

However, in western countries, this mustard oil is not considered suitable for consumption and has a reputation for being toxic. In certain countries, it is even sold as a massage oil, but again, is not considered edible. Whatever the reputation maybe, this Mustard Oil has a ton of health benefits and can be used quite safely by everyone.

1.3.3 Nutritional Value of Mustard Oil per 100 gm:

Mustard essential oil has all the nutrients of the mustard seed. In 100 grams of mustard oil, you will find 884 calories. It has a total fat content of 100 grams, including 12 grams of saturated fat, 21 grams of polyunsaturated fat, and 59 grams of monounsaturated fat. It has 0 mg of sodium in it along with 0 g of carbohydrates and proteins.

Mustard Oil does not contain any Vitamins and minerals that are essential to your body. However, mustard oil does contain a few vital chemical components in it. Mustard Oil contain fatty acids like oleic, linoleic, and erucic acid as well as an important substance called Allyl isothiocyanate, which contributes to its many health benefits.

Nutritional facts per 100 Grams in Mustard Oil:

884 Calories 100g Total fat



FIGURE NO: 3 Nutritional facts

1.3.4 Health Benefits of Mustard Oil:

Mentioned below are the best health benefits of Mustard Oil. You can start adding Mustard Oil in your dishes to get all these benefits since easily buy in your local markets. You can either use Mustard Oil in your cooking or you can apply directly to your skin can hair to get direct benefits.

1.3.5 Mustard oil benefits for blood circulation:

Mustard essential oil is used to boost circulation as it is a strong stimulant. This is extremely good for you because it provides oxygenated blood to your different organs at a much faster rate, improving their general function and productivity.

Mustard oil also promotes the secretion of gastric juices and bile from the liver and spleen, keeping your digestive system intact. The mustard oil also impacts peristalsis in the intestines, leading to better and more regular bowel movements.

1.3.6 Mustard Oil is an irritant:

While this may not sound like a benefit, it definitely is one. If your organs are numb or not functioning correctly, an irritant can be used as a stimulus to jump-start it, in a sense. It can show how well the organ responds to external stimuli and

whether or not it is in good condition. Moreover, the mustard oil is also used to stimulate the growth of muscles and to pump them up by exciting them.

1.3.7 Mustard oil boosts your appetite:

Mustard essential oil can be used by people who rarely feel hungry and are underweight or undernourished. This is because this oil can boost your appetite and make you want to eat more. This happens due to the fact that this oil is both, a stimulant and an irritant. Mustard Oil pumps up your stomach and also facilitates the secretion of gastric juices and bile, which creates the feeling of hunger in most people. Thus, it can encourage you to eat more by making you feel hungry.

1.3.8 Mustard oil fights against bacterial infections:

Mustard oil is known for fighting bacterial infections as well, as it has strong antibacterial properties. It works well on external and internal infections.

Externally, it can be applied topically on to a wound to prevent it from getting infected. Internally, it can help against infections that occur in the colon, urinary tract, digestive system, and the excretory system in general.

1.3.9 Mustard oil prevents fungal infections.

Mustard oil contains allyl isothiocyanate in it, which has strong antifungal properties. It can therefore prohibit the growth of fungus anywhere on the body. Furthermore, if you already are suffering from a fungal infection, you can use this mustard oil to kill the fungus and get rid of the infection.

1.3.10 Mustard oil repels insects:

Mustard oil is one such home remedy. It can be used effectively in vaporizers or fumigants to drive away any insects that might be present in your home or your surroundings, keeping you safe from their bites and diseases.

Insect bites can be extremely dangerous. You never what kind of acid an insect might be carrying and may secrete into your body when it bites you. This can lead to all sorts of health problems from dermatitis to malaria, which is why you should always be careful if there are insects present around you.

Mustard oil can be used to repel mosquitoes, many store-bought bug repellants contain chemicals that can have side effects if inhaled or exposed to your

skin. This is why a lot of people prefer home remedies, especially if they have to use the repellent on a regular basis.

1.3.11 Mustard oil benefits for hair growth:

In many cultures, using mustard oil is a vital part of hair care. Different essential oils contribute different benefits to your hair and are used accordingly. Mustard essential oil is also very good for your hair. Since it is filled with fatty acids like oleic acid and linoleic acid, it can be used to revitalize your dull hair.

It also has stimulating effects due to which it increases the circulation in the scalp, which helps it stay healthy. The hair roots are nourished well by the fatty acids in the mustard oil.

Mustard essential oil, if used on a regular basis on your hair, can prevent hair loss.

1.3.12 Mustard oil prevents phlegm:

Mustard oil is a cordial substance. This means that it brings warmth to your body. Mustard oil also warms up your entire respiratory system and prevents the formation and accumulation of excessive amounts of phlegm. As a result, it improves your breathing and can protect you from a number of breathing disorders like asthma and bronchitis.

Mustard oil stimulating effects keep the respiratory system healthy while its irritant effects work on the phlegm to keep it away. It is therefore a good oil to use if you suffer from congestion and breathing problems.

1.3.13 Mustard oil promotes sweating:

Sweating is an important body function. It helps you get rid of all the unwanted toxins in your body like ureic acid, excess water, fats, and salts. The buildup of these toxins can be quite harmful and can lead to many health problems. This is why inducing sweat through mustard essential oil is good for your system.

1.3.14 Mustard oil is a toner:

Mustard oil works as a toner. This means that it can tone up your entire system and keep it healthy. Mustard oil ensures that all your organs and systems run smoothly by stimulating the secretion of important hormones and enzymes.

Most hormones and enzymes are responsible for the way our body works and ensuring that they are secreted in the right amounts and at the right time can really have a huge impact on the positive functioning of your body as a

whole. Furthermore, mustard essential oil even helps you boost up your immune system, thus protecting you from a range of diseases and infections.

1.3.15 Mustard oil benefits for arthritis:

Mustard essential oil has been used since ancient times in order to provide relief from the symptoms of rheumatoid and arthritis. It can help with the pain that is caused by these problems when applied topically onto the joints that hurt. Moreover, the mustard oil can also help with the inflammation in these areas, bringing you a ton of relief.

Use Mustard oil to give massage to rheumatoid arthritis patients. This oil is very helpful and even you can add camphor to reduce pain and inflammation.

1.3.16 other health benefits of mustard oil:

Apart from the ones listed above, mustard oil provides a wide range of health benefits to your system. It can help you treat a variety of problems like headaches, cough, and cold, congestion that occurs due to coughs and cold, pain in the body, and even hampered muscle growth. You can rub this oil on your gums to strengthen them.

Uses of Mustard Oil:

Mustard essential oil has popular culinary uses in India and Bangladesh, where it is an essential part of the cuisine. It adds a unique flavour to the food. This oil is also used in massages due to the various benefits it leads to in the muscular system, for pain control, and even for the general circulation of blood in the body.

Mustard oil is rarely used in aromatherapy. This is because it does function as an irritant and therefore, does not have the calming effects that one desires during aromatherapy. It has been used in herbal and Ayurvedic medicine since ancient times and is proven to be quite useful for a number of different ailments.

1.3.17 Mustard Oil Side-Effects & Allergies:

Mustard oil has a reputation for being toxic, however, this is disproved by the pregnant women in India and Bangladesh that consume this oil on a regular basis and do not show signs of adverse effects on their children. It is even used to massage children to stimulate their growth and development. If you have extremely sensitive skin, or are vulnerable to allergies, you should definitely perform a patch test to see whether or not it gives

you an allergic reaction as this oil definitely shows the properties of an irritant. Mustard comes from the foothills of the Himalayas, and also grows naturally in Africa, the Middle East, and Mediterranean Europe. As a condiment, mustard is used all over the world, which is why finding mustard essential oil is also not particularly difficult no matter where you live.

You can easily find this oil at any shop that specializes in herbal medication.

II. CHAPTER-2

2.0 STANDARD DRUG PROFILE:

ALBENDAZOLE

Albendazole is an Anthelmintic (an-thel-MIN-tik) or antiworm medication. It prevents newly hatched insect larvae (worms) from growing or multiplying in your body. Albendazole is used to treat certain infections caused by worms such as pork tapeworm and dog tapeworm. Albendazole may also be used for purposes not listed in this medication guide.



Mechanism of action:

Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by diminishing its energy production, ultimately leading to immobilization and death of the parasite. It works by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. As cytoplasmic microtubules are critical in promoting glucose uptake in larval and adult stages of the susceptible parasites, the glycogen stores of the parasites are depleted. Degenerative changes in the endoplasmic reticulum, the mitochondria of the germinal layer, and the subsequent release of lysosomes result in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminth.

PHARMACOKINETICS:

Absorption: Poorly absorbed from the gastrointestinal tract due to its low aqueous solubility. Oral bio availability appears to be enhanced when co-administered with a fatty meal.

Distribution: 70% bound to plasma protein and is widely distributed throughout the body.

Metabolism: Liver

Elimination: Urine **Half-life:** 8-12 hrs **Side effects:**

- Stomach pain
- Nausea
- Vomiting
- Headache
- Dizziness
- hair loss

Uses: For the treatment of parasitic worm. This is used for the treatment of cystic hydrated disease of the liver and lung caused by dog tapeworm.

Dept of Pharmacognosy, Dr.KVSP, Kurnool.

III. CHAPTER-2

3.1 PLANT PROFILE:



FIGURE NO: 2 Brassica nigra flowers

Morphology of Brassica Nigra:

Synonym: Brassica perviridis, Brassica oleracea

Scientific classification:

Kingdom: plantae

Order: Brassicales

Family: Brassicaceae

Genus: Brassica **Species:** Brassica nigra

Synonyms:

English: Brown Indian mustard

White/ yellow mustard

Telugu: Avalu
Hindi: Raya

Macroscopic and Microscopic Examination of seeds:

The seeds were reddish brown with a smooth texture and approximately 0.9-1 mm in diameter; they had a bitter taste and characteristic pungent smell when crushed. The test was dark reddish-brown to yellow and minutely pitted. The cells of the outer epidermis of the tests contained mucilage. The embryo was oily and yellow in colour, containing two cotyledons folded against their midribs to enclose the radicals.

Physiochemical characterization of seeds:

The results obtained for the ash values and extractive values determined by methods described in ayurvedic pharmacopeia, can be used for the quality control purposes for mustard seeds, in various pharmacological interventions. The mean, range and standard error values of ash contents and extractive values of B.nigra seeds that resulted from analyses.

BOTANICAL REVIEW:

Common Name: Black Mustard

General Information:

Black mustard is an erect, annual plant, branched above, growing from 30 - 200cm tall, exceptionally to 300cm. The plant is often cultivated for its edible seed, though it is going out of favour because it rapidly sheds its seeds once they are ripe and this makes it harder to harvest mechanically than the less pungent brown mustard (Brassica juncea). The seed is used especially as food flavouring, though it is also sown with the seeds of garden cress (Lepidium sativum) to provide mustard and cress, a salading eaten when the seedlings are about one week old. Black mustard is also grown as a medicinal plant **Known**

Hazards

When eaten in large quantities, the seed and pods have sometimes proved toxic to grazing animals.

Botanical References:

Range




The original habitat is somewhat obscure, probably the Mediterranean region. Occasionally naturalized in S.W. Britain.

Habitat

Cliffs near the sea in S. W. England

Properties of black mustard

Table: 1

Weed Potential	Yes
Edibility Rating	
Medicinal Rating	
Other Rating	Uses 
Habit	Annual
Height	1.20 m
Pollinators	Bees, Flies
Self-fertile	Yes
Cultivation Status	Cultivated, Wild

Cultivation Details

Black mustard is adapted to a wide variety of climatic conditions. It is usually grown in the temperate and subtropical zones, being unsuited to wet tropical lowland areas but is able to be grown as a rained crop in tropical areas of low or moderate rainfall. The plant tolerates an annual precipitation of 300 - 1,700mm, an annual average temperature range of 6 to 27°C. An easily grown plant, black mustard is suited to many types of soils except very heavy clays, it grows best on light sandy loams, or deep rich fertile soils. Succeeds in full sun in a well-drained fertile preferably alkaline soil.

Edible Uses

Leaves - raw or cooked A hot flavour, they can be finely chopped and added to salads or cooked as a potherb. The seedlings can also be used as a salad, when about one week old, adding a hot pungent to salad. Immature flowering stems - cooked and eaten like broccoli. Mustard seed is commonly ground into a powder and used as a food flavouring and relish. This is the black mustard of commerce, it is widely used as a food relish and as an ingredient of curry. Pungency of mustard develops when cold water is added to the ground-up seed - an enzyme (myrosin) acts on a glycoside (sinigrin) to produce a sulphur compound. The reaction takes 10 - 15 minutes. Mixing with hot water or vinegar, or adding salt, inhibits the enzyme and produces mild bitter mustard. The seed can also be used whole to season pickles, curries, sauerkraut etc. Black mustard has a stronger more pungent flavour than white mustard (*Sinapis Alba*) and brown mustard (*B. juncea*) edible oil is obtained from the seed.

Medicinal Uses:



FIG NO: 4 Medicinal uses

Mustard seed is often used in herbal medicine, especially as a rubefacient poultice the seed is ground and made into a paste then applied to the skin. in the treatment of rheumatism, as a

means of reducing congestion in internal organs. Applied externally, mustard relieves congestion by drawing the blood to the surface as in head affections, neuralgia and spasms. Hot water poured on bruised seeds makes a stimulant foot bath, good for colds and headaches. Old herbals suggested mustard for treating alopecia, epilepsy, snakebite, and toothache. Care must be taken not to overdo it, since poultices can sometimes cause quite severe irritation to the skin. The seed is also used internally, when it is appetizer, digestive, diuretic, emetic and tonic. Swallowed whole when mixed with molasses, it acts as a laxative. A decoction of the seeds is used in the treatment of indurations of the liver and spleen. It is also used to treat carcinoma, throat tumours, and impostures. A liquid prepared from the seed, when gargled, is said to help tumours of the sinax. The seed is eaten as a tonic and appetite stimulant. Hot water poured onto bruised mustard seeds makes a stimulating foot bath and can also be used as an inhaler where it acts to throw off a cold or dispel a headache. Mustard Oil is said to stimulate hair growth. Mustard is also recommended as an aperients ingredient of tea, useful in hiccup. Mustard flour is considered antiseptic.

Agro forestry Uses

The seed germinates freely, and quickly, growing away rapidly and making a very useful green manure. The plant is often grown for this purpose, producing a bulk suitable for digging into the soil in about 8 weeks. It does harbour the pests and diseases of the cabbage family, though, so is probably best avoided where these plants are grown in a short rotation and especially if club root is a problem.

Other Uses

Semi-drying oil is obtained from the seed, as well as being edible it is also used as a lubricant, illuminate and in making soap [Mustard oil (allyl isothiocyanate) is used in commercial cat and dog repellent mixtures.

IV. CHAPTER-4

4.0 AIM AND OBJECTIVES:

AIM:The aim of the present study was to investigate the anthelmintic activity of mustard seeds (*Brassica nigra*) using adult earthworms.

OBJECTIVES:

1. Collection and authentication of *Brassica Nigra* seeds

2. Successive solvent extraction of the drug
3. Phytochemical screening of extracts
4. Evaluation of Anti-helmentic of Brassica Nigra

V. CHAPTER-5

5.0 METHODOLOGY:

5.1 Collection of plant materials: The plant Brassica nigra seeds were collected from surrounding regions of Dupadu hilly area of Kurnool district dried under shade and crushed to a coarse powder.

5.2 Extraction of Drug:

The dried powdered plant material of Brassica nigra was extracted with ethanol using soxhlet extraction method.

5.3 Drugs and Chemicals:

Solvents and chemicals used during experimentation were of analytical grade and obtained from drug store of Dr. K. V. Subba Reddy Institute of Pharmacy.

5.4 Earthworms:

Adult Indian earthworms (*Pheretima Posthuma*) were obtained from vermicompost, Kurnool, and washed with normal saline to remove all the fecal matter. The earthworms are of 3-5cm in length and 0.1 -0.2 cm in width was used in all the experimentation.



Fig 8: MUSTARD SEEDS Fig 9: PULVERIZATION OF SEEDS



Fig 10: PULVERIZATION OF POWDER

5.5 SOXHLET EXTRACTION METHOD:



Fig 11: Soxhlet extraction of Brassica Nigra seeds

Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor several times. The process involves extraction followed by evaporation of the solvent. Then vapour of the solvent are taken to a condenser and the condensed liquid is returned to the drug for continuous extraction.

Soxhlet apparatus designed for such continuous extraction consist of the body of the extractor attached with a side tube and siphon tube as shown in figure. The extractor from the lower side can be attached to distillation flask and the mouth of the extractor is fixed to a condenser by standard joints. The crude drug powder is packed in the soxhlet apparatus directly or in a thimble of filter paper or fine muslin. The diameter of the thimble corresponds to the internal diameter of the soxhlet extractor.

Extraction assembly is set up by fixing a condenser and a distillation flask. Initially for the setting of the powder, the solvent is allowed to pass through siphon once before heating. Fresh activated porcelain pieces are added to flask to avoid bumping of the solvent. The vapour pass through the side tube and the condensed liquid gradually increases the level of liquid in the extractor and in the siphon tube.

A siphon is set up as the liquid reaches the point of return and the content of the extraction chamber are transferred to the flask. The cycle of solvent evaporation and siphoning back can be continued as many times as possible without changing the solvent, so as to get efficient. This

method although a continuous extraction process, is nothing but a series of short macerations.

Similar methodology can be adopted in large scale production in which the operation principle may resemble the laboratory equipment. Soxhlet extraction is advantageous in a way that less solvent is needed for yielding more concentrated products. The extraction can be continued until complete exhaustion of the drug. The main disadvantage is that this process is restricted to pure boiling solvent or to azeotropes.

5.6 PHYTOCHEMICAL SCREENING:

Detection of Alkaloids:

➤ Wagner's test:

Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of reddish brown or brown ppt indicated the presence of alkaloids.

➤ Dragendroff's test:

Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red ppt indicated the presence of alkaloids.

➤ Hager test:

Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow ppt indicates presence of alkaloids.

➤ Mayer's Test:

Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of yellow cream ppt indicates the presence of alkaloids.

Detection of Flavonoids:

3ml of each extract was added to 10ml of distilled water and the Solution was shaken. 1ml of 10% NaOH solution was added to the Mixture.

Detection of Saponins:

➤ Frothing test:

3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.

Detection of Steroids

➤ Salkowski Test :

5 drops of concentrated H₂SO₄ were added to 1ml of each extract in a separate test tube.

Detection of Tannins:

➤ Ferric chloride test:

The extract was treated with few drops of neutral ferric chloride solution 5%. The formation of bluish black colour indicated the presence of tannins.

➤ Gelatin test:

To the extract, 1% gelatin solution containing sodium chloride was added. The formation of white ppt indicated the presence of tannins.

➤ Lead acetate test:

The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow ppt indicated the presence of tannins.

Detection of Glycosides:

➤ Legal's test:

The extracts were treated with sodium nitro prusside in pyridine and methanolic alkali. The formations of pink to red colour indicate presence of glycosides.

➤ Balget test :

The extract of drug was treated with sodium picrate and the formation of yellowish orange colour confirmed the presence of glycosides.

Detection of Reducing Sugars:

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.

Volatile oils:

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl.



FIGURE 12: phytochemical screening

VI. CHAPTER-6 6.0 RESULT AND DISCUSSION:

The seeds of *Brassica nigra* belonging to family (Brassicaceae) were selected for the study. The plant material was collected from local areas of Dupadu Kurnool dist and authenticated by Vanitha kumari Dept of Botany, HOD of St. Joseph's Degree College, in Kurnool.



Table: 2 Result of Phytochemical screening:

S.NO	Name of phytoconstituent	Brassica nigra seed ethanolic extract
1.	Alkaloids	+
2.	Glycosides	+
3.	Flavonoids	+
4.	Tannins	+
5.	Steroids	+
6.	Volatile	+
7.	Reducing sugar	+

Colour, nature and percentage yield of the extracts:

Name of the plant: *Brassica nigra*

Weight of powdered seeds: 60 gm

Extracts: ethanolic extract

Colour: yellowish colour

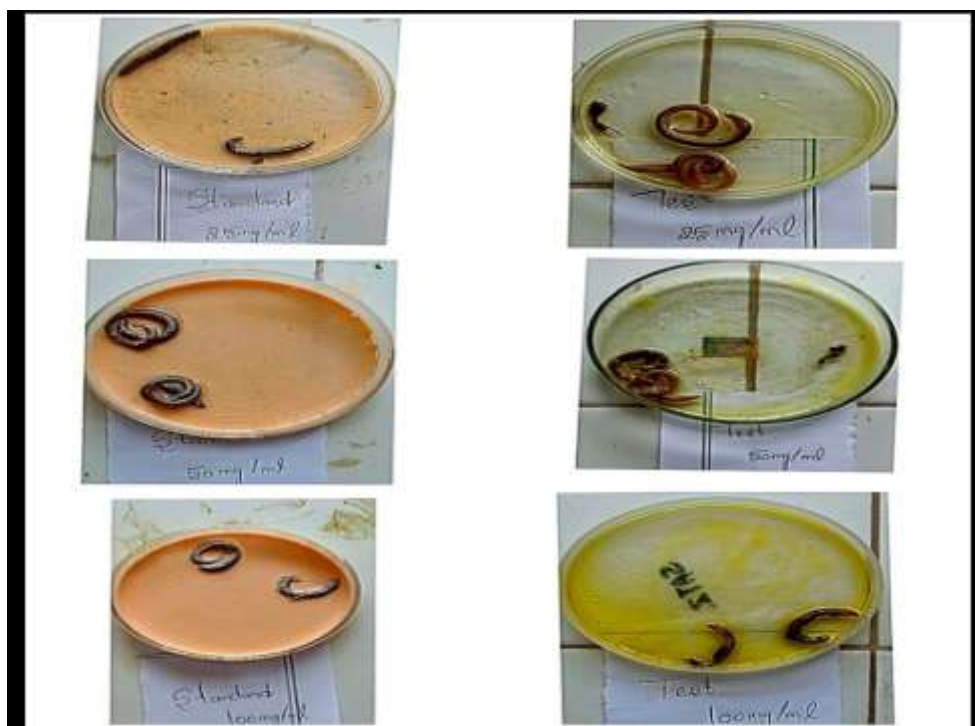


FIG NO.13: AntiHelminthic activity of different concentrations of *Brassica nigra* ethanolic extract and Albendazole.

TABLE NO: 3

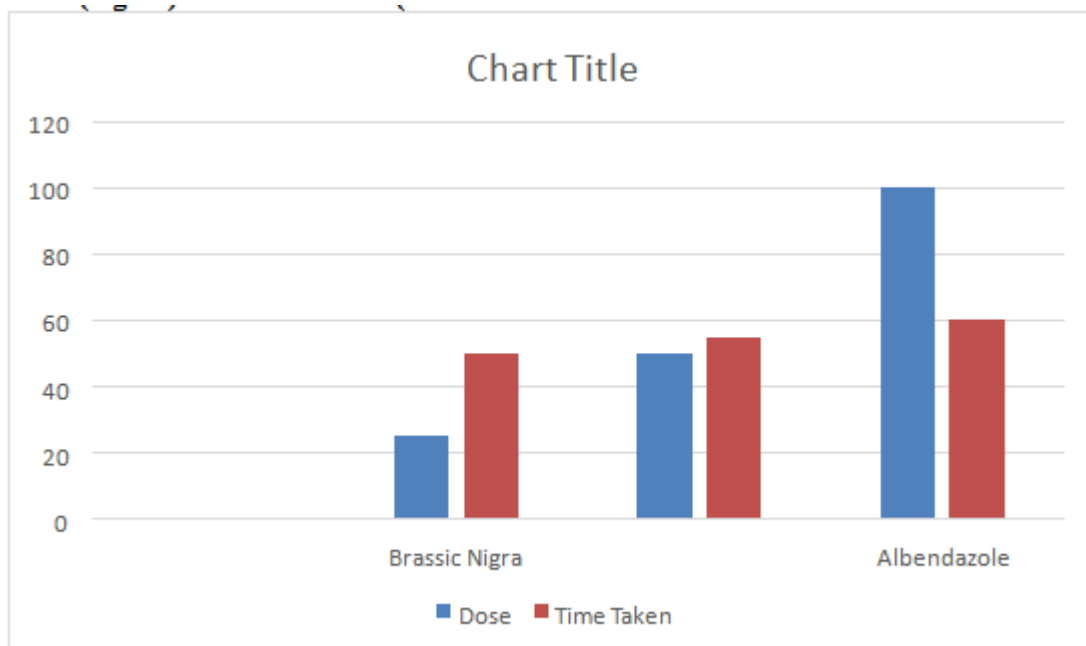
S.NO	Type of extract	Dose(mg/ml)	Time taken(min)
1.	Brassica nigra	25	30
		50	55
2.	Albendazole	100	60

Dose (mg/ml) and Time taken (min)

TABLE NO.4

S.NO	TYPE OF EXTRACT	DOSE(mg/ml)	TIMETAKEN(min)
1.	Brassica nigra	25	50
		50	55
2.	Albendazole	100	60

Dose (mg/ml) and Time Taken (min)



Graph:.1.Antihelmintic activity-death of earthworm at different concentration seed extract of brassica nigra and compared with the standard Albendazole respectively.

TABLE NO.5

S.NO	TYPE OF EXTRACT	DOSE(mg/ml)	TIMETAKEN(min)
1.	Brassica Nigra	25	55
		50	60
2.	Albendazole	100	68

Dose (mg/ml) and Time Taken(min)



Graph 2: Anti hermitic activity-death of earthworm at different concentration seeds extract of Brassica Nigra