JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Investigations on Ziziphus Jujuba for Pharmacognostical and Anthelmintic Activity -- A Pilot Study

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Abstract:

Ziziphus jujuba Mill. commonly called jujube (Rhamnaceae) has been known for its health benefits and used to cure different diseases such as Anthelmintic , asthma, cough and anxiety. The aim this research work deals with the detailed pharmacognostical evaluation of the crude drug. Morpho anatomy of the entire plant have been studied with the aim to aid pharmacognostical and taxonomic species identification using WHO recommended physico-chemical determinations and microscopical characters that will provide referential information for checking adulteration. The physico-chemical and histological parameters presented in this paper may be proposed as parameters to establish authenticity of *Ziziphus Jujuba* and can possibly help to differentiate the drug from its other species. Many important diagnostic characters such as bicollateral vascular bundle, paracytic stomata, pitted and spiral vessel, presence of stone cells, starch grains, lignified xylem and phloem will certainly help in identification of drug. As there is no pharmacognostical anatomical work on records for this traditionally valued herb, the present study is taken up in the view to lay down the physico-chemical and microscopic standards. Microscopical and physicochemical standards discussed here can be considered as the identifying parameters to substantiate and authenticate the drug and could be useful in the preparation of herbal monograph for its evaluation. Medicinal plants were the potent source of many pharmacological activities. Among those the plants of anthelmintic action has attained a great interest due the capability of the plant and its compound to treat a disease that causes major economic loss and reduced livestock production to the livestock holders. Helminthiasis causes a significant health

problem with increased morbidity and, to some extent, mortality in an underdeveloped and developing country, although it may also occur in developed countries. It remains undiagnosed in many patients, and they suffer a lot due to many complications. This activity reviews the evaluation and treatment of helminthiasis and highlights the role of the interprofessional team in evaluating and treating patients with this condition.

Keywords: Ziziphus jujube, Anthelmintic Activity,

1. INTRODUCTION

The development of traditional medicinal systems incorporating plants as means of therapy can be traced back to the Middle Paleolithic age some 60,000 years ago as found from fossil studies. In recent times, developed countries are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies18 and according to the World Health Organization (WHO), almost 65% of the world's population has incorporated the value of plants as a methodology of medicinal agents into their primary modality of health care. It is often noted that 25% of all drugs prescribed today come from plants. This estimate suggests that plant-derived drugs make up a significant segment of natural product based pharmaceuticals.

Fig. 1 Fruits of Ziziphus Jujuba

Fig. 2 Leaves of Ziziphus Jujuba

Medicinal plants were the potent source of many pharmacological activities. Among1 that the plants of anthelmintic action has attained a great interest due the capability of the plant and its compound to treat a disease that causes major economic loss and reduced livestock production to the livestock holders. The pathogenic infection causes the severe effect of mortality and other problems that were uncontrolled due to the anthelmintic resistance that is developed in the host organism. Even though, many synthetic drugs were manufactured; they produce more side effect than that of the treatment efficacy. Hence, the need for the exploration of the plants for the treatment has attained a great interest and this review gives the list of few medicinal plants that were capable of reducing the helmintic infection.

Traditional herbal medicine, as a natural source, plays an important role in treatment of various diseases and maintenance of health. Traditional treatments that were used in various traditional medical schools have been repeatedly examined by a number of great physicians through experiments and clinical trials over thousands of years. Even now, hundreds of years later, traditional medicine has not lost its value. Z. jujuba, a small deciduous tree or shrub, is widely cultivated around the world and its ripe fruit is used as food and medicine since ancient times. The various parts of this plant such as fruits, leaves, roots and seeds have medicinal value. Z. jujuba is beneficial for the treatment of different diseases, such as asthma, constipation, cough, inflammation, insomnia, anxiety, laryngitis as well as heart, liver and kidney diseases6. Various chemical constituents from different phytochemical classes such as alkaloids, polysaccharides, flavonoids are found in Z. jujube fruit. For example, spinosin (Scheme 1), flavones C-glycoside, was isolated from Z. jujuba seed.

Spinosin at 5 mg/kg exerts its anxiolytic-like effects by modulation of γ-aminobutyric acid-A (GABAA) and 5-hydroxytryptamine-1A (5-HT1A) receptors compared with diazepam as a control group in mice. In an in vivo study the sub chronic administration of spinosin (5 mg/kg), exhibited significant increase in the proliferation and survival of neuronal cells and the number of immature neurons in the hippocampus dentate gyrus region. Spinosin in dose dependent manner enhance phenobarbital induced sleep by increasing sleep time and reducing sleep latency via a serotonergic mechanism11. In addition, due to the presence of carbohydrates, oral administration of aqueous extract of Z. jujuba fruit is a beneficial treatment for constipation.

2. Helminthes and Classification of Helminthes.

Helminthes word is derived from the Greek“meaning worm. Helminthes are referred to various types of parasitic worms that reside in the body(bhatia). The helminthes include the flatworms (flukes and tapeworms) which arecovered by the plasma membrane and the Nematoda or roundworms , these are covered by tough cuticle.

A. Flukes (Trematodes)

These are covered by plasma membrane, these are bisexual. The life-cycle includes a snail intermediate host.

B. Tapeworms (Cestodes)

Adult tapeworms are elongated, segmented, hermaphroditic flatworms that inhabit the intestinal lumen. These are covered by tough cuticle.

C. Roundworms (Nematodes)

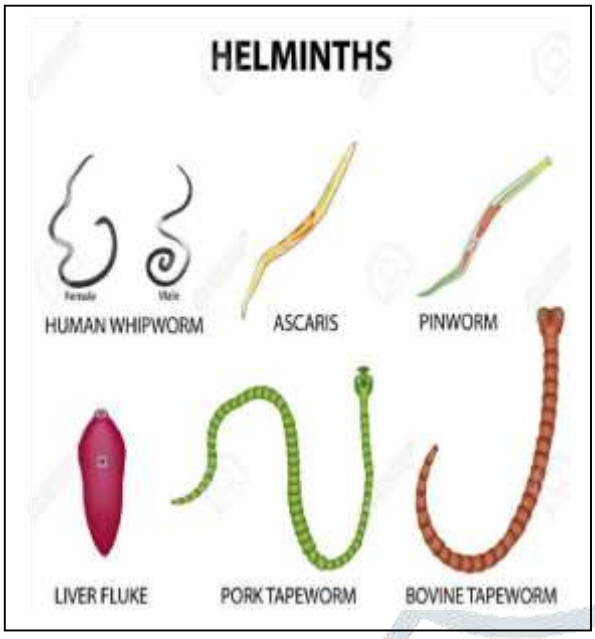
Adult and larval roundworms are bisexual, cylindrical worms. They inhabit intestinal and extra intestinal sites. These are covered by tough cuticle.

Screening of anthelmintic activity

The worms used in the in vitro assay are

- Pherethima posthuma
- Ascardia galli
- Ascaris lumbricoids

- Raillietina spiralis



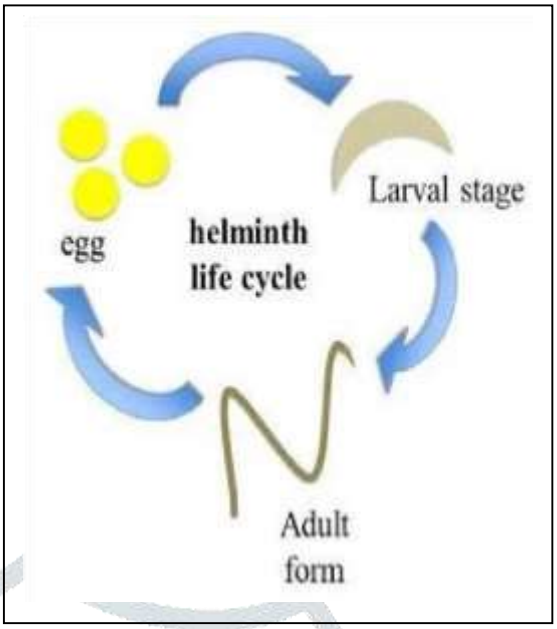


Fig. 3 Types of Worm		Fig.4 Life Cycle of Worm

The word "helminths" comes from the Greek meaning worm. The parasites that infect humans can be classified as heirlooms or souvenirs. Parasites that are inherited from ancestors in Africa are called Heirlooms, and those that are acquired from the animals during contact through our evolution, migrations, and agricultural practices are called souvenirs. In developing countries, the most common infectious agents of humans are these helminthic infections. More than a quarter of the world's population, that means approximately 2 billion people are affected by the helminthic parasite, and it is one of the major burdens of developing countries, especially in children. There are two major phyla of helminths known as nematodes and platyhelminths. Nematodes are also known as roundworms that include soil-transmitted helminths and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis. Other phyla platyhelminths also called flatworms, which include flukes schistosomes and tapeworms such as the pork tapeworm that causes cysticercosis. Flukes are known as trematodes, and tapeworms are known as cestodes. Soiltransmitted helminthiasis is a roundworm (Ascaris lumbricoides), whipworm (Trichuris trichiura), and hookworm (Ancylostoma duodenale and Necator americanus). The soil-transmitted helminths (STHs), enter into the human body from contaminated soil that contains eggs of A. lumbricoides and T. trichiura. Some helminth can penetrate the skin directly (hookworm larvae). The diseases by helminths are neglected tropical diseases because they usually have insidious effects on growth and development. Also, the study of these diseases receives less than 1% of the global research budget.

3. Etiology's of Helminthiasis.

Intestinal parasite infections often cause morbidity and mortality, especially in children. The major risk factors of helminthiasis are rural areas, low socioeconomic status, poor sanitation, poor availability of clean water, poor personal hygiene, lack of nail trimming, crowded living conditions, lack of education, lack of access to health care, and inadequate dwelling conditions.

A. A. lumbricoides and T. trichiura are transmitted through the fecal-oral route. Adult Ascaris is a long cylindrical worm, and its larvae can migrate into the pulmonary circulation, but T. trichiuria cannot.

B. A. duodenale and N. americanus are transmitted by penetration of the skin from where it goes into the lungs and crosses pulmonary capillaries to penetrate into alveolus and then to the intestine through the passing of larynx. N americanus is globally predominant compared to A. duodenale. S. stercoralis can infect percutaneously and orally.

C. Poor hygiene of mother or caregiver is also one of the most important risk factors for soil-transmitted helminths infection in preschool children.

D. Schistosomiasis infection is usually transmitted from contact with freshwater snails during swimming or washing. Schistosomiasis causes chronic inflammation that produces oxygen-free radicals. These free radicals are responsible for different mutations and the formation of carcinogenic N-nitrosamines that cause bladder carcinoma and portal tract fibrosis.

Fig. 5 Etiology of Worms' infection

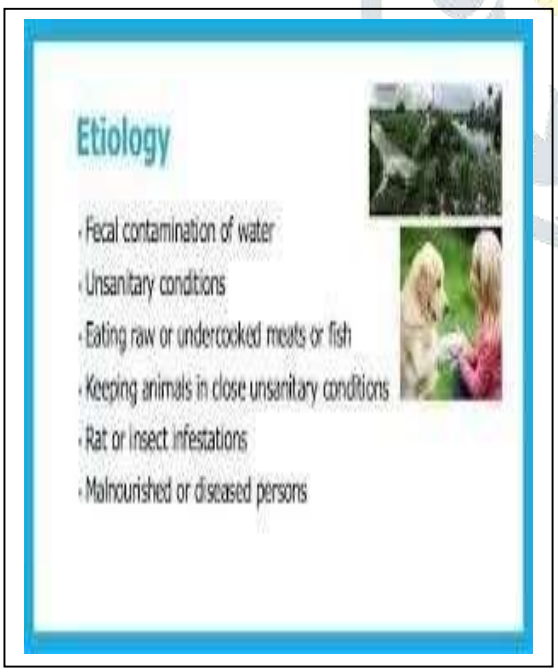
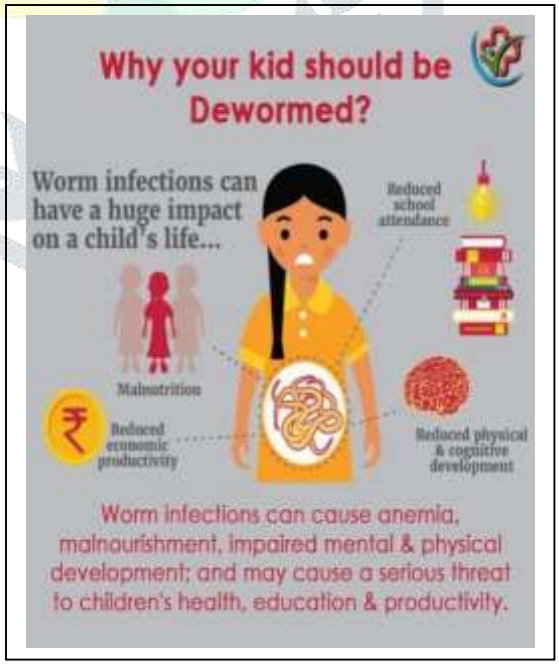


Fig. 6 Effect of worms on child Health



E. Diphyllobothriasis is most commonly occurs by species being D. latum from the ingestion of larva of the fish tapeworm.

4. Medicinal Uses of Ziziphus Jujuba

There are large numbers of traditional medicinal uses that are not necessarily based on knowledge of the constituents. According to Ayurveda, the plant of *Ziziphus Jujuba* is bitter and cooling, and cures coughs, biliousness and headache. The bark cures boils and is good for the treatment of dysentery and diarrhea. The leaves are antipyretic and reduce obesity. The fruit is cooling, digestible, tonic, aphrodisiac, laxative and removes biliousness, burning sensations, thirst, vomiting and is also good in treating tuberculosis and blood diseases. The seeds cure eye diseases and are also useful in leucorrhoea.

Fig. 7 National Deworming Day

5. Collection of plant material

The plant leaves of *Ziziphus Jujuba* were collected from available graphical sources. The plant drugs were identified, collected and stored for further use.

Preparation of plant material

The collected *Ziziphus Jujuba* plant was washed with tap water. The plant leaves were crushed into small pieces and air-dried thoroughly under shade (at room temperature) for 1 month to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight container for furthers analysis.

6. Determination of Physicochemical Parameters of selected plant

A. Determination of total ash

Two grams of the whole plant powder of the *Ziziphus Jujuba* was placed in a previously ignited (350°C for 1 hour) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550°C for 5 hours in a muffle furnace until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material.

B. Determination of acid-insoluble ash

Twenty- five (25) ml of hydrochloric acid (~70g/l) TS was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550°C for 3 hours in a muffle furnace to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acidinsoluble ash content was calculated as mg per g of air dried material.

i. Determination of water-soluble ash

Twenty- five (25) ml of water was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes. Insoluble matter was collected on an ash less filter- paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water-soluble ash content was calculated as mg per g of air-dried material. **ii. Determination of sulfated ash**

Ignited a suitable crucible (silica) at 550°C to 650°C for 30 minutes, cooled the crucible in a desiccator (silica gel) and weighed it accurately. One gram of the plant powder of the *Ziziphus Jujuba*, was placed in a previously ignited crucible, ignited gently at first, until the substance was thoroughly white. Cooled and moistened the sample with a small amount (usually 1 ml) of sulfuric acid TS, heated gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moistened the residue with a small amount (usually 1 ml) of sulfuric acid TS, heated gently until white fumes were no longer evolved, and ignited at 800°C + 25°C until the residue is completely incinerated. Ensure that flames were not produced at any time during the procedure. Cooled the crucible in desiccators, weighed accurately. This was repeated until the sample reaches a constant weight and calculated the percentage of residue.

C. Preparation of plant extracts

Collected moderately coarse plant powder of *Ziziphus Jujuba* was used for the preparation of various extracts. The plant leaves powder of the *Ziziphus Jujuba* was extracted with petroleum ether, methanol and water using as solvent respectively by continuous hot extraction. The residue was

evaporated by filtration through Whatmann No. 1 filter paper and the aqueous extract was concentrated used on a Rotary evaporator to get solid yield extract.

Plant name	Solvents for extraction
Ziziphus Jujuba	Petroleum ether
Ziziphus Jujuba	Methanol
Ziziphus Jujuba	Water

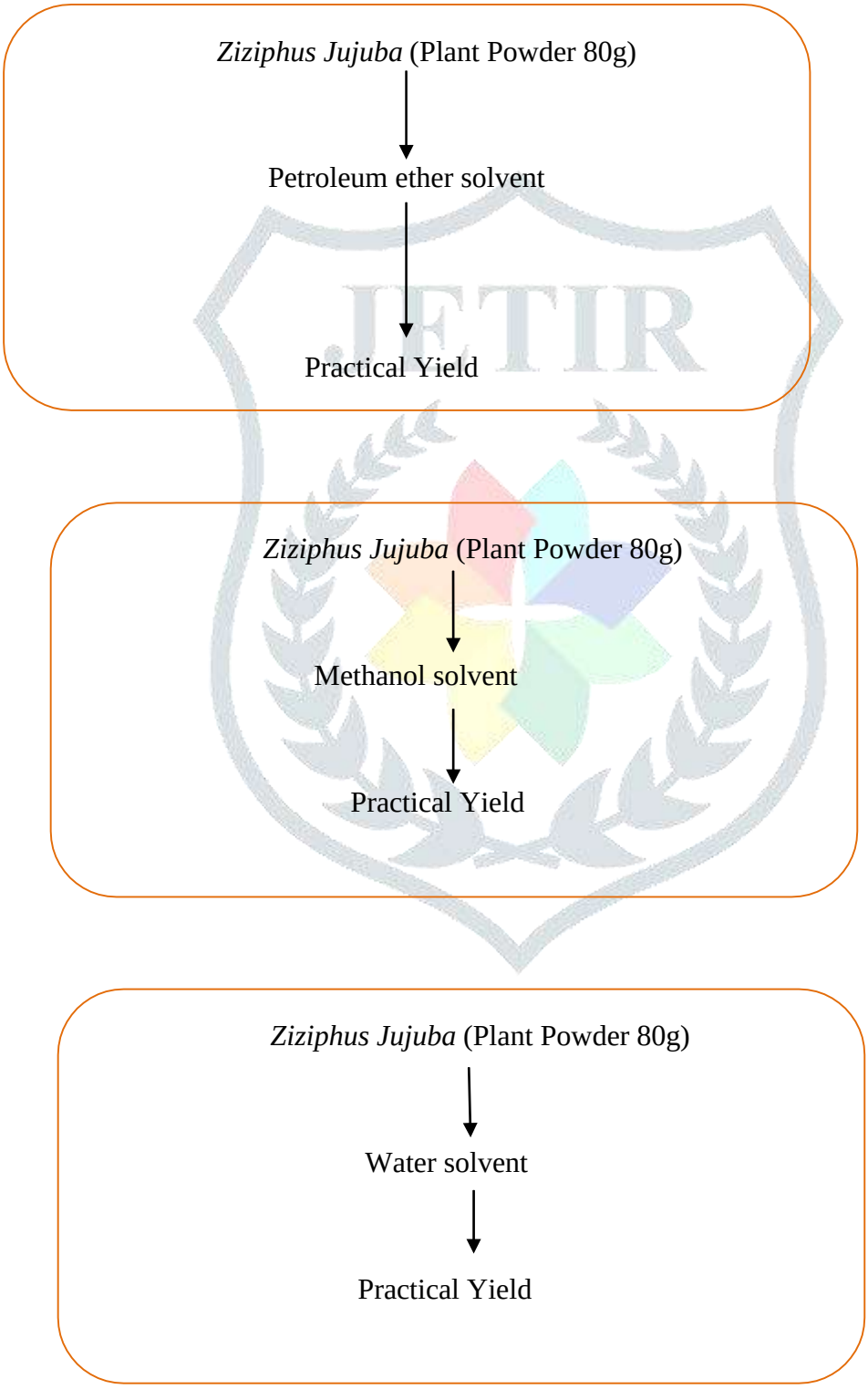


Fig. 6 Extraction protocol of selected plants

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. It refers to the extraction, screening and identification of the medicinally active substances found in plants. The preliminary phytochemical screening of the ethyl acetate, methanol and water extracts of plant powder of *Ziziphus jujuba* was carried out using standard laboratory procedures to detect the presence of different secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, coumarins, reducing sugars, protein, fixed oils and fats.

Following phytochemical tests of plant extracts were performed.

0.5g of each extract was stirred with 5mL of 1% aqueous Hydrochloric acid on a steam bath. 1mL each of the filtrate was treated, and then it was treated separately with a few drops of Dragandroff's reagents, Meyer's reagent and Wagner's reagents. End colour was noted.

i. Test for Cardiac Glycosides (Keller-Killiani test):

0.5g of each extract was dissolved in 3mL of Ferric chloride in glacial acetic acid and leave for a minute. 15mL of concentrated sulphuric acid was added with the aid of pipette, so that it runs down the side of the test tube.

ii. Test for Flavonoids

1mL of each extract was dissolved in 2mL of sodium hydroxide solution. The appearance of a yellow solution which disappeared on addition of Hydrochloric acid indicates the presence of flavonoids. **iii. Test for Saponins:**

10mL of distilled water was added to 0.5mL of each extract. Shake the content vigorously with the test tube for 2 minutes. The presence of frothing or bubbling indicates the presence of saponins. **iv. Test for Steroids:**

5 drops of concentrated sulphuric acid was added to 1mL of the extract. A reddish brown colour indicates the presence of steroids.

v. Test for Tannins (Ferric chloride Test):

A little portion of extract was diluted with water in the ratio of 1:4 and few drop of 10% Ferric chloride solution was added, end colour was noted which indicated presence of tannins. **vi. Test for Terpenoids**

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey color formed which showed the entity of terpenoids.

7. Evaluation of Anthelmintic activity of plant extracts

The plant extracts of *Ziziphus Jujuba* were evaluated for anthelimintic activity in Pheretima posthuma (earth worm) of nearly equal size (6±1 cm). Pheretima posthuma is used due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human begins. Because of easy availability of earthworms, they have been used widely for the initial evaluation of the anthelmintic compounds. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into five groups of six earth worms in each and placed in eight Petri dishes containing the extract solutions or the reference drugs as mentioned below;

i. Group -1: Received distilled water which served as the control

ii. Group-2: Received Albendazole suspension at a dose of 10mg/ml which served as the standard

iii. Group-3: Received Petroleum ether extract at a dose of 100mg/ml

iv. Group -4: Received Methanolic extract at a dose of 100mg/ml

v. Group-5: Received Aqueous extract at a dose of 100mg/ml

All Petri dishes were kept under room temperature. The living or viable worms were kept under close observation. Observations were made for time taken to complete paralysis (PT) and death (DT) for individual worms. Each worm was frequently applied with external stimuli which stimulates and induce movement in earthworms, if alive. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading of the body colour.

8. RESULTS AND DISCUSSION

A. Physicochemical Investigations

Physicochemical parameters were determined as per guidelines of WHO, air dried coarse powdered sample of *Ziziphus jujuba* were subjected for determination of physicochemical parameters such as pH, foreign organic matter, methanol soluble extractives, water soluble extractives, total ash content, acid insoluble ash, water soluble ash, loss on drying and % moisture content were determined. The Average physicochemical parameters of the *Ziziphus jujuba* course powder are tabulated in table.

B.Extraction of plant Drug

The plant leaves powder of the *Ziziphus jujuba* was extracted with ethyl acetate, methanol and water using as solvent respectively. The solvent was removed and practical yield was found and recorded. The findings were tabulated in table.

a. Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethyl acetate, methanol and water extracts of plant powder of *Ziziphus Jujuba* were carried out using standard laboratory procedures to detect the presence of different secondary metabolites. All the findings were recorded in table. **Table: Physicochemical Parameters of *Ziziphus Jujuba plant***

Parameters	Values
Total ash value	4.20±1.25
Water soluble ash	1.20±0.10
Acid insoluble ash	1.45±0.40
Sulphated ash	1.70±0.10

Table. Extractive values of *Ziziphus Jujuba*

Solvent	Yield (g)	% Yield
Petroleum ether	8.2g	10.25%
Methanol	13.5g	16.87%
Water	11.0g	13.75%

Table: Preliminary phytochemical screening of petroleum ether extract of *Ziziphus jujuba*

Phytoconstituents	Pet ether extract
Alkaloids	Negative
Glycosides	**Positive**
Flavonoids	Negative
Saponins	Negative
Steroids	**Positive**
Tannins	Negative
Terpenoids	Negative

Table: Preliminary phytochemical screening of methanol extract of *Ziziphus jujuba*

Phytoconstituents	Methanol extract
Alkaloids	Negative
Glycosides	Negative
Flavonoids	**Positive**
Saponins	Negative
Steroids	Negative
Tannins	**Positive**
Terpenoids	**Positive**

Table: Preliminary Phytochemical screening of aqueous extract of *Ziziphus jujuba*

Phytoconstituents	Aqueous Extract
Alkaloids	Negative
Glycosides	Negative
Flavonoids	**Positive**
Saponins	**Positive**
Steroids	Negative
Tannins	**Positive**
Terpenoids	**Positive**

Table: *In-vitro* anthelmintic activity of different extracts of *Ziziphus jujuba*

Groups	Concentration mg/ml	Time taken for Paralysis (in mins Mean)	Time taken for Death (in mins Mean)
Control (Distilled water)	-------- -	00	00
Standard (Albendazole)	100	28±0.25	57±0.20
Pet ether extract	100	39±0.25	70±0.10
Methanolic Extract	100	29±0.25	62±0.30
Aqueous Extract	100	32±0.25	65±0.50

CONCLUSION:

The pharmacognostical study is a major and reliable criterion of identification of plant drugs. The physico-chemical parameters are necessary for confirmation of the identity and determination of quality and purity of crude drugs. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years there has been an emphasis on standardization of medicinal plants, and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. Physico- chemical studies on different plants have been done by various workers. According to World Health Organization (WHO) the macroscopic and microscopic

description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

The physico-chemical parameters help in judging the purity and quality of the drug. The powder drugs were evaluated for its physico-chemical parameters like foreign matter, loss on drying, total ash, acid insoluble ash and different extractive values. The current study establishes not only physicochemical characterizations of plants but also phytochemical characters of all the plant extracts. These characteristics can be used further as identification and authentication parameters of the plant extracts. All the plant extracts are found to be rich in flavonoids and saponins having wide spectrum of bioactivity. The plants studied here can be seen as a potential source of useful therapeutics. Further studies are going on these plant extracts in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with their pharmacological activity. In other words, the physicochemical features examined in the current study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations at herbal industrial level in the coming days.

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 based drugs. Plant materials evaluated in the current study had been identified from various sources to serve as anthelmintic agents by traditional healers of Ethiopia. The *in vitro* tests using free living stages of parasitic nematodes offer a means of evaluating the anthelmintic activity of new plant compounds. *In vitro* techniques are preferred to in vivo methods due to their low cost, simplicity, and rapid turnover. In the current study, a statistically significant association was noted between graded concentrations of the extracts, the exposure test-time interval, and adult parasite mortality. Activity was evaluated by noting the time required for paralysis and death of worms by extracts. The findings showed that methanolic extract (100 mg/ml concentration) possess comparable anthelmintic activity with standard drug. The results show that the plant has the potential to be used as anthelmintic. Therefore, further study must be carried out so that the general people can get

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