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REVIEW ON IN-VITRO EVALUATION OF ANTIHELMITINIC ACTIVITY ON MEDICINAL PLANTS

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Abstract : Helminthiasis is the most common infection caused by worms that is contaminant to human body parts. Normally, the worms live in the gastrointestinal tract, liver and other organs. The currently available anthelmintic drugs, including albendazole, mebendazole, thiabendazole, niridazole, dietylcarbamazine, ivermectin, praziquantel, are widely used to control helminthiasis. But these drugs have serious drawbacks such as hepatotoxicity, loss of appetite, dizziness, nausea, vomiting, abdominal pain, headache and diarrhoea. Thus, it is necessary to look for more effective anthelmintic drugs with the minimum side effects. Eighty percent of the world's population relies on traditional medicines and plant extracts and the active constituents are used to meet people's primary health care needs. This review focuses on helminthiasis and the role of traditional plants in the treatment of helminthiasis.

Index Terms : **Anthelmintic drugs, Albendazole, Mebendazole, Thiabendazole and Niridazole.**

I. INTRODUCTION :

Modern synthetic medicines are very effective in curing diseases but also cause a number of side effects. Crude drugs are less efficient with respect to cure of diseases but are relatively free from side effects. Parasites have been of concern to the medical field for centuries and the helminths considered causing considerable problems for human beings and animals. A large number of medicinal plants are claimed to possess anthelmintic property in traditional systems of medicine and are also utilized by ethnic groups worldwide. Following the folk claims, several medicinal plants have been scrutinized for this activity using various *in vitro* and *in vivo* methods. The present review summarizes some important pharmacological and preliminary studies on medicinal plants, products thereof and isolated principles from them, which can be investigated further to achieve lead molecules in the search of novel herbal drugs.

Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials, antihelmintics etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. The use of chemical anthelmintics drugs for controlling animal parasite is rapidly losing popularity due to a number of disadvantages. Anthelmintic resistance in the parasites is spreading and the inefficacy of chemical anti-parasitic compounds is threatening animal health. New plants with medicinal properties against parasites of ruminants are being investigated around the world with promising results. In the near future natural products obtained from plants extracts seems that likely will become a viable alternative of control of parasitizes of veterinary importance. WHO has recently estimated that 80% of the populations of the developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs? In India, the history of medicinal uses of plants dates back to 3500-1800 B.C. where in the Rig-Veda mentions a number of plants with different healing practices. A large part of the population depends even at the present time on the indigenous systems of medicine, ayurveda, Unani and Sidha. Now days a number of plant showing antiparasitic properties and use as potent antiparasitic agent. The aqueous and alcoholic extracts of Ananas sativus (Bromeliaceaea), Embellia ribes, Macuna prurita (Leguminosae) and Melia azedarach have been found to bear significant activity against Taenia canina and Paramphistomum cervi; M. prurita, in particular, has been found to be more effective against trematodes. The plant extract of papaya possess a dose dependedent effect on larva and adult worm of T. colubriformis (Hounzangbe et al., 2005) [29]. The aqueous, etheral and alcoholic extracts of Cucurbita mexicana (Cucurbitaceae) seeds have exhibited significant anthelmintic activity against Moniezia expansa, Fasciolopsis buski, Ascaris lumbricoides and Hymenolepis diminuta. Fumaria

parviflora ethanol extract eliminated fecal eggs and caused 72 and 88% mortality of adult Haemonchus contortus and Trichostrongylus colubriformis, respectively. The anthelmintic activity of ethanolic extract of Melia azedarach Linn. (Meliaceae) was found to be better against T. solium than that of piperazine phosphate (Szewczuk et al., 2003) [71]. Recent surveys in developing countries have identified many plants that are intended have the potential to be used as anthelmintics.

II. ADVANTAGE OF PLANT BASED ANTHELMINTICS OVER CHEMICALS ANTHELMINTICS :

1. Synthetics anthelmintics are expensive whereas plant based anthelmintics are less expensive.

2. Synthetics anthelmintics cause drug residues problem while plant based anthelmintics are free from drug residues.

3. There is chance of drug resistance after prolonged use of synthetics anthelmintics whereas in plant based anthelmintics have less chance of drug resistance.

4. Synthetics drugs are unavailable in rural areas whereas it is easily available.

5. Synthetics drugs cause environment pollution whereas plant based anthelmintics are eco-friendly and promote biodiversity.

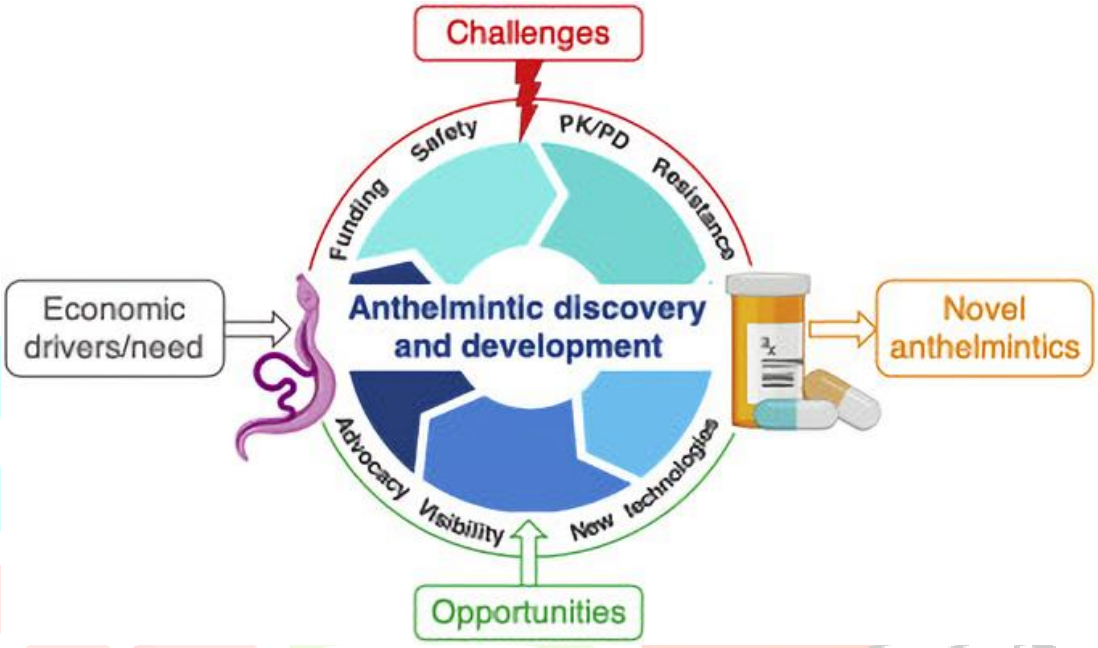

Figure 1: Anthelmintic discovery and development

III. COMMON PHYTOCHEMICALS FOUND IN PLANT CONTAINING POTENT ANTHELMINTIC ACTIVITY :

a. Alkaloids e.g. Palasonin

b. Isoflavones e.g. Genistein

c. Triterpenoids e.g. Ursolic acid

d. Polyphenols (Tannins and flavonoids), simple phenols (Phenolic acids)

e. Saponins

f. Organosulfides- Allicin, Isothiocyanates,

g. Thymoquinone

h. Cysteine proteinases

IV. METHOD OF EXTRACTION OF PHYTOCHEMICALS :

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal (Das et al, 2010).

V. CAUSES OF ANTIHELMITINIC DRUGS:

- Headache
- Abdominal pain
- Nausea
- Vomiting
- Dizziness
- Vertigo
- Hair fall
- Fever
- Rash
- Anemia
- Acute liver failure

VI. EXTRACTION PROCEDURES :

VII. PLANT TISSUE HOMOGENIZATION:

Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and dissolved in the solvent to determine the concentration. Serial exhaustive extraction It is another common method of extraction which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (Methanol) to ensure that a wide polarity range of compound could be extracted. Some researchers employ soxhlet extraction of dried plant material using organic solvent. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds (Das et al, 2010). c. Soxhlet extraction Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds (Nikhal et al, 2010). d. Maceration In maceration (For fluid extract), whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs (Ncube et al, 2008). e. Decoction This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume (Remington, 2008). f. Infusion It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water (Remington, 2008). g. Digestion this is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased (Remington, 2008). h. Percolation this is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (A narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 hr.

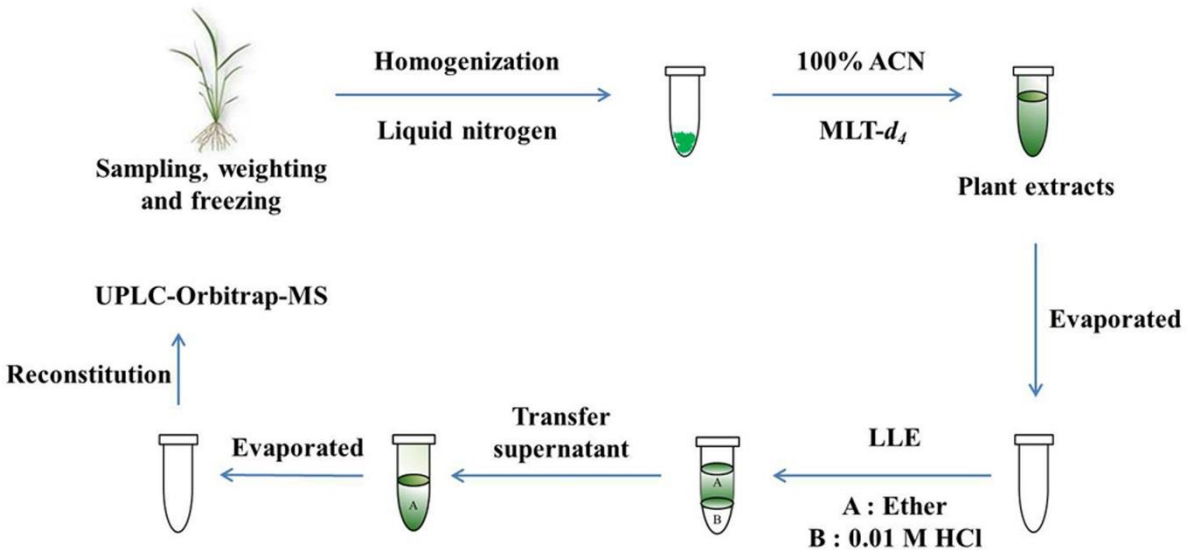

Figure 2 : PLANT TISSUE HOMOGENIZATION

VIII. SCREENING OF PHYTOCHEMICALS :

Phytochemical examinations were carried out for all the extracts as per the standard methods. 1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. a. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. b. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids. c. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate. 2. Detection of glycosides Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. a. Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides. b. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides. 3. Detection of Saponins a. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. b. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

IX. INVITRO STUDY :

In the beginning, most of the in vitro researches regarding anthelmintic activity of plants, their different extracts or oils have been based on their toxic effects on earthworm, Pheritima posthuma (Gaind and Budhiraja, 1967; Ali and Mehta, 1970; Kokate and Varma, 1971; Dixit and Varma, 1975; Banerjee and Nigam, 1978; Girgune et al., 1978; Agarwal et al., 1979; Girgune et al., 1979; Mishra et al., 1979; Mehta et al., 1981; Garg and Kasera, 1982a, b; Dengre, 1982; Nanda et al., 1987; Siddiqui and Garg, 1990; Garg and Siddiqui, 1992). Most of these substances which are toxic to earthworms produce a primary irritation or agitation that results in the withdrawal of the worm from the neighborhood of the poison. By asset of this effect, anthelmintics doubtless often drive out the parasite when the concentration does not get sufficiently higher to kill the worm (Sollmann, 1918). Some workers have also used hookworms, Haemonchus contortus, and tapeworms and/or Ascaris lumbricoides for the evaluation of in vitro anthelmintic tivity of different plant materials (Dubey and Gupta, 1968; Sharma et al., 1971; Kalesaraj, 1974, 1975; Dixit and Varma, 1975; Banerjee and Nigam, 1978; Girgune et al., 1978; Agarwal et al., 1979; Girgune et al., 5 1979; Mishra et al., 1979; Sharma et al., 1979; Shrivastava, 1979; D'Cruz et al., 1980; Mehta et al., 1981; Garg and Kasera, 1982a, b; Dengre, 1982; Kakrani and Kalyani, 1984; Kalyani et al., 1989; Siddiqui and Garg, 1990; Nakhare and Garg, 1991; Garg and Siddiqui, 1992; Garg and Jain, 1992). A modified egg hatch assay (Coles et al., 1992) is often used to evaluate the effect of plant products against eggs of Haemonchus contortus or other trichostrongylids. Some other researchers conducting in vitro studies have used an alteration of the larval development assay (LDA) or larval motility tests which are commonly used for testing of resistance of parasites to anthelmintics (Menezes et al., 1992; Nirmal et al, 1998; Al- Qarawi et al., 2001; Alawa et al., 2003; Assis et al., 2003; Lateef et al., 2003). The anthelmintic activities of different plants reported in literature for their in vitro anthelmintic activity have been tabulated/reviewed.

X. CONCLUSION :

The most prominent worm-borne infection that contaminates human body parts is helminthiasis. The worms typically reside in the liver, gastrointestinal system, and other organs. The majority of helminthiasis cases are managed using anthelmintic medications that are currently on the market, such as albendazole, mebendazole, thiabendazole, niridazole, dietylcarbamazine, ivermectin, and praziquantel. However, these medications have significant side effects, including hepatotoxicity, appetite loss, lightheadedness, nausea, vomiting, headaches, and diarrhea. Finding anthelmintic medications that are more effective while minimizing side effects is therefore imperative. Eighty percent of the world's population gets their primary healthcare from traditional medicines and plant extracts, which contain active ingredients. The focus of this review is on helminthiasis and how traditional herbs can be used to cure it.

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XII. CONFLICT OF INTEREST :

Authors declare no conflict of interest

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