Available Online through
www.ijpbs.com

International Journal of Pharmacy and Biological Sciences (eISSN: 2230-7605)
IJPBS |Volume 1| Issue 3 |JULY-SEPT |2011|198-230

ANTHELMINTIC ACTIVITY OF *SYMPLOCOS RACEMOSA*

NARASIMHA RAO R[1], BHAVYA B[2], PAVANI K[3], SWAPNA A[4], PRASOONA CH[5]
[1,2,3,4&5] **HITS COLLEGE OF PHARMACY, BOGARAM (V), KEESARA (M), R.R (DIST)-501301**
***Corresponding Author Email: rnrao007@yahoo.com**

Research Article

RECEIVED ON 19-07-2011 **ACCEPTED ON 05-08-2011**

ABSTRACT

In recent time there is an increase in global utilization of herbal medicine in the treatment of various decease affecting human. The highly safety profile and low cost of herbal medicines have been reported as the major factors responsible for the increased upsurge in herbal medication. The subject of the phytochemical analysis, phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and its closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function .At the present work Collection of plant material,Extraction of the crude drug, Successive solvent extraction ,Phytochemical tests of plant extract ,Thin layer chromatography, HPTLC , Invitro anthelmenthic activity.

***KEY WORDS**: Symplocos Racemosa, phytochemical analysis, Invitro anthelmenthic activity, , HPTLC, Thin layer chromatography*

Introduction[1]

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts are used as prescription drugs and their sales in the countries of European Union were around $ 6 billion in 1991 and may be over $ 20 billion now. In USA, herbal drugs are currently sold in health food stores with a turnover of about $ 4 billion in 1996 which is anticipated to double by the turn of the century.

The human being exploited to alleviate his suffering from injuries of deceases utilizing plant g rowing around him. The plant kingdom still hold many species of plant containing substance of medicinal value which have yet to be discovered and the large no. of plant are constantly being screened for their possible pharmacological value in addition to already exploited plants. As the results of modern isolation technique and pharmacological screening procedure, new plant drugs usually find their way into modern medicines. Now a days maximum world' population depends on herbal medicines.

Medicinal plants often contain additional active principles other than the major active principles and physiologically inert substances like cellulose and starch. Unlike the chemical entities, which contains one active ingredient pulps a number of inert substances, which makeup the dosage form (like tablet, capsules and syrups). Indian system of medicines

comprises of Ayurveda, Unani, Siddha, Homeopathy, Naturopathy, and Yoga. Each of which uses the herbal constituents in some or the other form, crud drug are not so effective because they have not been tested for efficacy according to rigid pharmacological standards. As the constituents derived from the medicinal plants proved the cure the medicinal plants proved to cure the human disorders they isolated and used for their pharmacological action [6-14]

The constituents having particular therapeutic effect are identified and isolated. Natural product research has lead to a new physiological and pharmacological concept, particular when a new compound is found to have specific biological effects. Unlike synthesis drugs, which are synthesized in laboratory under the controlled conditions; the drug which are obtained from natural sources are showing variability in concept of active constituents due to one or more of the following reasons : Seasonal variability, Soil requirements, Altitude, Rain fall, Light, Temperature, Time of collection

During the 40 years precise method for the evolution of herbal pharmacopoeia drug & their preparation have been devised based on their source constitution derived from wild and cultivated origin.

In this respect internationally several pharmacopoeia have been provide monograph stating quality parameter and standardization of many herb and some product made out of these herbs. In India, the herbal drug market is about $ one billion and the export of plant-based crude drugs is around $ 80 million.

In recent years W.H.O has emphasized the need to ensure standardization of medicinal plant product by using modern techniques. In some case thought the chemical nature of active constituents is not possible to isolate without changing its chemical integrity then only way to left quality assurance chemist is to standardize it biologically.

General protocol followed for the standardization of herbal materials are mainly authentication, i.e. proper botanical identity, foreign matter content ie free from soil, stones, dust, insects and other contaminant, organoleptic evaluation, microscopial evaluation, volatile matter, ash value and extractive value and other protocol are like sampling preliminary examination, moisture content, loss of drying, swelling index, Rf values, microbial contamination toxic remedies etc. A Pharmacognostical study of a plant drug enables the scientist for proper identification of the drug and removes adulteration and provides a basis for authentication of the crude drug. The phytochemical investigation of a plant may involve the extraction of plant materials, separation and isolation of the constituents of the interest, characterization of isolated compounds. The chemist produces several new compounds every year as potential drugs. The pharmacologist numerous ways of testing these compounds in various animal sepsis and upon to make use of his experiences and inventiveness to limit the test of those likely to be the most rewarding the existing situation. This necessitates the development of a "**screening program**" for initial detection of more qualification as well as classification of biological activity. In standardization or evaluation of herbal drugs, assessment of biological efficacy is found to be most assuming method .In this method requirements are a suitable animal for testing and control, methodology for experiment assessment of result.

Herbal drugs have great growth potential in the global market. Natural product research continues to explore Indian Traditional Medicines to develop new novel drugs. In this Project I have focused on the strategies,

significance, guidelines and the research methods to be followed in order to develop herbal medicines which will gain international acceptance. Chromatographic fingerprinting techniques are most significant methods which can be used for the routine herbal drug analysis and for quality assurance. The WHO guideline parameters are discussed and some in vitro models for antioxidant studies, cytotoxic studies, are given in flow chart form in order to provide simple and effective assessment of the biological screening of botanicals without animal models or extensive extraction and purification steps.

Plants have a long history of medicinal use. In countries such as India and China they are a central part of medicinal treatment. Herbalism is the use of plant material, based on tradition and folk lore to treat or prevent disease. A herbal practitioner (or herbalist) treats by selecting a herb or combination of herbs specific for the set of symptoms of a particular patient. Herbal medicine (phytotherapy, phytomedicine) uses standardised extracts prepared from plants or plant parts for which there is documentation of therapeutic activity India is one of the 12 mega biodiversity centres having 45, 000 plant species; its diversity is unmatched due to the 16 different agroclaimatic zones, 10 vegetative zones, and 15 biotic provinces. The country has a rich floral diversity (Table 1). Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Traditional preparation comprises medicinal plants, minerals and organic matters etc.

Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record is evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years. About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments, which also forms a rich source of knowledge. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat

In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases. Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient

Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The prevalence of helminth diseases in Nigeria is very high, especially during the wet season when infection is as high as 100% in cattle. Such high infection rates prevent them from attaining optimum productivity, especially under the traditional husbandry system. Financial costs of internal parasitism are enormous due to increase in mortality and a reduction of growth rate and wool production. The major control strategy adopted against helminth parasites in Nigeria is the use of anthelmintics. However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelmintics has led to development of resistance and hence a

reduction in the usefulness of available anthelmintics. Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites, and as a means of reducing the cost of controlling helminthic diseases, the emergence of resistant strains of pathogenic helminth has stimulated the desire to search for additional chemotherapeutic agents that might allow more efficient control of helminth parasites. A practical solution to this is to develop effective drugs from reasonably less expensive and available raw materials. This can rationally be approached through the study of indigenous traditional plant remedies. In Nigeria, herbal treatment of helminthiasis is widely practised by human herbalists and the nomadic Fulani's (major cattle rearers in Nigeria) (NWUDE and IBRAHIM, 1980).

Given the world wide interest in exploiting the anthelmintic activity of plants or their products it is understandable that there is also a growing interest in finding the best ways of screening for bioactivity. However before embarking upon any screening program it is very important that researchers have clearly defined goals, which incorporate the needs of their end users since these will influence both the breadth and depth of their research projects. For example in a situation where there are only a small number of local forages that need investigation it might be possible to meet the anthelmintic activity needs of resource poor farmers through results generated in simple faecal egg count reduction tests using naturally infected animals. On the other hand it would be impossible to extend this *in vivo* approach to determine the efficacy of products derived from whole plants or their products to even moderate sized botanical collections containing a few hundred different species. The time and financial costs involved in all mass screening programs highlight the importance of the choice of first phase screening technique which needs to be sensitive, cheap and reproducible. Having used first phase screening to reduce the numbers of candidates then the next phases in the process of focussing in on the active compounds are inevitably more complex and exp ensive simply because as the degree of refinement increases so do the costs of discovery as one needs to add pharmacology, compound target and safety/toxicity data to the compound profile. Understanding the nature of the bioactive components, their mode of action and their targets within the parasite are all important in the processes leading to practical application. Bioactive plants may contain large numbers of plant secondary metabolites that may act singly or in combination to produce direct (Atha nasiadou *et al*., 2001) and /or indirect effects on parasites in the alimentary tract, leading to reduced nematode survival, growth and fecundity. They can also provide improved protein availability in the host and/or have direct or indirect effects on mineral or trace element status. Both of these changes to host nutrition can lead to reductions in parasite establishment, burden and fecundity through improvements in host immunoregulatory capacity.There are thought to be a number of active biochemical compunds that act against parasites including essential oils, proteolytic enzymes, lectins and polyphenolics such as the tannins (Hoste *et al* 2006). Since the focus of this paper is intended to be on the initial phases in the screening process it will not deal with the screening processes that are required to define the chemistry of the active compounds nor their pharmacology and toxicology.

ANTHELMINTIC DEFINITION: Pertaining to a substance that destroys or prevents the development of parasitic worms, such as filariae, flukes, hookworms, pinworms,

roundworms, schistosomes, tapeworms, trichinae, and whipworms. An anthelmintic drug may interfere with the parasites carbohydrate metabolism, inhibit their respiratory enzymes, block their neuromuscular action, or render them susceptible to destruction by the host's macrophages. Drugs used in treating specific helmintic infections include piperazine, pyrantel pamoate, pyrvinium pamoate, mebendazole, niclosamide, hexylresorcinol, diethyl carbamazine, and thiabendazole.

Before, the administration of a toxic anthelmintic it is customary to starve the patient for from twelve to twenty-four hours and to give a brisk cathartic, the object being to clean out the intestines and leave the worm in an exposed condition. The dose is then administered, and is followed in four or five hours by a brisk, rapidly acting cathartic, such as castor oil or salts, to carry out the worm. Castor oil has been objected to on the ground that an oily medium will promote the absorption of the poison by the patient. This may be true, especially in the case of oleoresin of male fern, if rapid evacuation of the bowels does not take place. The different kinds of parasite require different kinds of treatment, as follows:

1. The Pin-Worms Or Thread-Worms (Oxyuris Vermicularis): These are tiny, thread-like organisms which live in great abundance in the colon or the adjoining portion of the ileum, chiefly in the mucus. As they do not cling to the intestinal wall, they are readily carried out by cathartics; or, as they are very vulnerable, may be attacked by colon irrigations. Occasionally they penetrate the mucous membrane of the intestine or inhabit the appendix, and then they cannot be dislodged.

The cathartics mostly employed are calomel and castor oil. By mouth both thymol and oil of chenopodium, as used for hookworms, have proved highly effective. A number of substances are used for colon injection, viz., the infusion of quassia, lime-water, a solution of phenol, 0.25 per cent., a solution of quinine bisulphate, 1: 2000, a solution of tannic acid or alum, 30 grains (2 gm.) in one pint (480 c.c.), and soapsuds containing 1/2 ounce of the oil of turpentine to a quart. The astringents are effective not only by shriveling the worms, but also by lessening the intestinal mucus in which the worms may lodge. The Hymenolepis or Taenia nana, which are tiny tape-worms, are sometimes taken for pin-worms.

2. The Round-Worms:

a. The common round-worm, Ascaris lumbricoides, grows to a length of 6 to 12 inches or even more. They usually inhabit the small intestine, but may be found in the colon or stomach, and have been known to stop up the common bile-duct. The author has had several patients who have vomited round-worms, and in two instances drew up a piece of round-worm through a stomach-tube. These must have been in the stomach. They may be the cause of intestinal hemorrhage. The remedies are:

Santonin (santoninum), a glucoside from santonica (Levant wormseed), dose, 2 grains (0.12 gm.) for an adult, and 1 grain (0.06 gm.) for a child of five years. Santonica, 1/2 dram (2 gm.), is sometimes taken as it is or in the form of an infusion. Santonin is highly toxic, and death has occurred from 5 grains (0.3 gm.) in an adult, and 3 grains (0.2 gm.) in a child. The symptoms of poisoning are nausea, vomiting, and central stimulation. The reflexes are increased, and there may be headache, dizziness, delirium, hallucinations, and possibly epileptiform convulsions, followed by collapse and death. A peculiarity of santonin poisoning is partial blindness, accompanied by yellow vision. Baxter reports lost vision in a girl of five

after 1/2 grain (0.03 gm.). Jelliffe (1906) reports prolonged convulsions, followed by collapse, in a girl, from two troches followed by castor oil which failed to move the bowels. After this she was blind, very restless, and prostrated for three weeks, and showed signs of nephritis. She became a permanent epileptic.

The treatment of poisoning is lavage of the stomach, followed by a large dose of Epsom salts, the inhalation of ether, and the management of symptoms as they arise. The central stimulation must be handled with care because of the tendency to collapse. Santonin has come into notice of late as a remedy for the pains of locomotor ataxia and for diabetes, but clinical data do not justify these uses of so dangerous a drug.

Spigelia (pink-root) has an official fluidextract, dose, 60 minims, (4 c.c.). It is frequently given with senna (fluidextract of pink-root and senna), the senna furnishing the required, though rather late, catharticaction. In poisoning it causes central depression, with prostration, stupor or coma, muscular weakness, incoordination, and collapse.

b. The hookworms (Uncinaria or Necator or Ankylostoma americana) are treated by thymol or oil of chenopodium. Thymol - Public Health Bulletin No. 32 recommends a dose of Epsom salts at night, followed at 6 A. M. by half the dose of thymol, at 8 by the other half of the thymol, and at 10 by another dose of Epsom salts. This treatment is repeated once a week. The dose recommended is 7 1/2 grains (0.5 gm.) for a child of five years, and 45-60 grains (3 to 4 gm.) for an adult, given in 5-grain (0.3 gm.) capsules. It is best mixed with an equal weight of lactose or sodium bicarbonate. Seidell finds insignificant amounts of thymol in the feces and only 50 per cent. in the urine, He notes that absorption is not promoted by its solution in oil. Thymol has also been employed in trichinosis, both while the parasites are still in the intestine and when they are lodged in the muscles. For the latter, 2 to 3 grains (0.12-0.2 gm.) in 30 to 45 minims (2-3 c.c.) of olive oil are injected subcutaneously daily. Musgrave recommends thymol for irrigation in amebic colitis. Thymol has in several instances caused fatal poisoning of the volatile oil type. Death has resulted from 15 grains (1 gm.) in a child; yet in adults.

DRUGS USED FOR TREATMENT: 1. Mebendazole , 2. Albendazole, 3. Thiabendazole ,4. Pyrantel pamoate , 5.Piperazine 6. Niclosamide ,7. Metronidazole ,8. Metrifonate ,9. Ivermectin, 10. Praziquintel

PLANT PROFILE- LOHDRA BARK[2]

BOTANICAL NAME *Symplocos Racemosa*

FAMILY SYMPLOCACEAE

SUBSTITUTE *SYMPLOCOSPANICULATA*
SYMPLOCOS CRATAEGOIDES

IMAGES OF SYMPLOCOS RACEMOSA

OTHER NAMES

• SANSKRIT	»	*Aksibhaisajya, Aksibhesaja, Balabhadra, Balipriya, Bhillataru, Bhilli, Galava, Hastilodhraka, Hemapushpaka, Kandakilaka, Kandanila, Laktakarma, Lodhra, Lodhrah, Lodhraka, Lodhravriksha, Mahalodhra, Marjana, Nayanousadha, Rodhra, Rodhrah, Savara, Savaraka, Savarakarodhra, Savararodhra, Savura, Shahara, Shaharalodhra, Shambara, Shavaraka, Shukla, Srinata, Sthulavalkala,*
• HINDI	»	*Lodhra, Lodhra pathani*
• BENGALI	»	*Lodhra*
• MARATHI	»	*Lodhra*
• URDU	»	*Ludhpathani, Pathan lodh, Lodh pathani, Lodh pathani sayida, Pathani lodh*
• TAMIL	»	*Vellattippattai, Vellilottiram, Velli-lodhram, Kacacankai, Kaya vilai*
• TELGU	»	*Lodduga, Lodhuga-chettu, Sabaramu, Sapara, Erralodduga*

DESCRIPTION

a) **Macroscopic:** Trees or shrubs, usually glabrous. Leaves often turning yellow when dry, alternate, coriaceous or membranous, toothed or en'ire. Flowers are usually white, in axillary spikes or racemes, sometimes reduced to few-flowered fascicles or to a single flower; bracts usually solitary at the base of each pedicel, caduceus; bracteoles are 1-3 beneath the flower. Calyx-tube is adnate, short when in flower, often enlarged in fruit; lobes 5, imbricate. Petals 5 in 1 series, or 6-10 in 2 series, free almost or entirely to the base, or obscurely connate (rarely connate into a tube), imbricate. Stamens are usually numerous, many seriate, adnate to the corolla-tube or to the petals, the outer the longer; filaments

filiform or flattened at the base; anthers short dehiscing longitudinally. Ovary inferior (in Indian species), 3- (rarely 2- or 4-) celled; ovules 2, pendulous from the inner angle of each cell; style usually filiform; stigma capitate or small, scarcely lobed. Drupe is ellipsoid or subglobose; stone usually woody, often ribbed, 1-3-seeded. Seeds oblong; embryo terete, straight or curved, in the centre of fleshy albumen; cotyledons much shorter than the radicle [Mhaskar et al., 2000].

Mature stem bark occurs in channelled or curved pieces, few fiat pieces also occur in thickness upto 1cm, outer surface uneven and rough due to fissures and cracks, grayish brown to grey externally, pale to whitish-brown internally, fracture short and granular in cortical region and somewhat fibrous in inner region, taste, astringent and feebly bitter.

b) Microscopic : Transverse section of mature bark shows a wide cork of thin-walled, rectangular cells arranged in radial rows, cork cambium 1-3 layered, secondary cortex consists of thin-walled, oval and tangentially elongated parenchymatous cells towards outer side and rounded cells towards inner side, a number of stone cells, in singles or in groups present, scattered throughout the region having highly thickened walls with distinct pits, prismatic and cluster crystals of calcium oxalate, and starch grains, mostly simple present in a number of cortical cells, secondary phloem wide consisting of sieve elements, phloem parenchyma, phloem fibres and stone cells, phloem parenchyma thin walled, oval to rectangular, containing prismatic crystals of calcium oxalate scattered in phloem parenchyma, phloem fibres lignified and present in singles or in groups, crystals not present in fibres, isolated fibres spindle shaped with pointed ends, groups of stone cells as rounded patches distributed throughout phloem region, medullary rays uni to multiseriate consisting of rectangular cells having brown colouring matter in some cells, broader medullary rays dialating towards outer phloem region, a number of phloem cells also contain starch grains, mostly arranged in groups, rarely solitary, simple and rounded. Powder-Greyish-brown, under microscope shows fragments of cork, stone cells, fibres, prismatic and cluster crystals of calcium oxalate and starch grains.

DISTRIBUTION : It is large genus of trees and shrubs, widely distributed in the tropics and subtropics of Asia, Australia, and America. It consists of almost 290 species, about 68 species are found in India, of which only a few are of economic importance. In Pakistan only two species are found, namely *Symplocos chinensis* and *Hymplncos racemosa* [Chadha., 1976; Mhaskar *el* a/., 2007]

FORMULATIONS (Yog)	Abhrak bhasma	Asthisandhanaka lepa	Bhringaraj taila
	Briha gangadhara	Chandanasava	Dashmularishta
	Drakshadi kvath chuna	Gangadharchurna (vrihat)	Grahanimihir taila
	Irimedadi taila	Jatyadi taila	Jivantyadi ghrita
	Kasisadi ghrita	Khadiradi gutika (mukharo	Kumaryasava (a)

	Kunkumadi taila	Kutajastak kvath churna	Laghugangadhara churn
	Lodhrasava	Nagarjunanjan	Nyagrodhadi churna
	Pippaladyasava	Piyushvalli rasa	Prameha mihira taila
	Pushyanug churna	Rodhrasava	Sarivadyasava
	Somnath ras	Srikhandasava	Tutthadi lepa
	Ushirasava	Vastyamayantaka ghrita	Vidangarishta
	Vimla vartti		
DOSE	Churna 1 - 3 gm, Kwath 5 - 10 ml		
CHEMICAL CONSTITUENTS	Monomethyl pelargonidin glucosides, Loturine, Colloturine, Loturidine, Oxalic acid, Phytosterol, 3-monoglucofuranoside, Betulin, Acetyloleanolic, Oleanolic, Ellagic acid		
PHYSICAL CONSTITUENTS	Total ash 12 %, Acid insoluble ash 1 %, Alcohol soluble extractive 9 %, Water soluble extractive 15 %		
AYURVEDIC PROPERTIES			
• **GUNA (Quality)** • **RASA (Taste)** • **VIPAK(Metabolism)** • **VIRYA (Potency)** • **PRABHAV(Impact)**	• Laghu, Ruksha • Kashay • Katu • Sheet • Aartav-sangrihniya		

MEDICINAL IMPORTANCE: *Symplocos racemosa* (Lodh) is a medicinal plant widely used by the traditional practitioners against various diseases as single or in compound drug. It has a wide range of usage in Ayurveda and Unani medicines. Its bark is described as an emmenagogue tonic for the persons of plethoric constitution and is useful in bowel complaints and ulcers. Its decoction is used as a gargle for giving firmness to bleeding and spongy gums. It cures watery eyes, opthalmia and is good for all diseases of the eye. It also cures "Kapha" biliousness, diseases of the blood, dysentery, inflammations, vaginal

discharges, leprosy, elephantiasis, filaria, and is useful in abortions, miscarriages and ulcers in the vagina. The bark in 20-grain doses mixed with sugar, is given in menorrhagia due to relaxation of the uterine tissue; it should be given two or three times a day, for three or four days. It is also used for leucorrhoea. The bark is also prescribed in the treatment snake-bite and scorpion-sting. In snake bite it is given internally in powder form or in the form of a decoction [Joshi., 2000; Mhaskar *et al.,* 2000].

Screening methods for anthelmintics[3] :Test parasite should be taxonomically and biologically close to target parasite in humans. Screening models in suitable small animal hosts.

1. Antiheliminticactivity:

In vitro methods: Adult worms: - Litomosoides carinii, Acanthocheilonema viteae. Onchocerca gibsoni Setaria cervi Microfilareae: -L. carinii, Dirofilaria, Conspiculum guidense, S. cervi

(1) Setaria cervi : Whole worm preparation Adult S. cervi (Nematoda: Filarioidea) obtained from peritoneal cavity of the freshly slaughtered cattle Transported to the laboratory in a vacuum flask containing modified Ringer's solution, (NaCl-9g, CaCl2-0.24g, KCl- 0.42g, NaHCO3- 0.5g,Glucose-0.25g/Litre) 20 ml capacity isolated organ bath containing modified Ringer's solution at 37% used for suspending the worm.

Posterior end tied to hook at the bottom of the bath. The anterior end was attached to a frontal writing lever. At least 15 min allowed for a worm to stabilize Drug added in increasing conc., movements recorded on slow moving drum. Drug -- inactive if it does not modify movements in 10 minutes. Fresh worm used to test each dose of a drug [15-22]

Nerve – muscle preparation: Worm placed in petri dish containing modified ringer (370 C). 2 dissecting needles inserted at one end, cuticle split longitudinally. Intestine, uterus removed. Ant. 1 cm of worm removed to eliminate influence of nerve ring, cephalic ganglia Remaining part tied, suspended, tested as above.

(2) Onchocerca gibsoni: A primary in vitro screen was developed to screen for drug activity against Onchocerca volvulus. Assay estimates variation in motility through motility meter. Results compared favorably with reported in vivo tertiary screens for activity against Onchocerca species. Effects of these drugs were not reversible. Thus reduction in motility regarded as indicating significant metabolic damage.

(3) Acanthocheilonema viteae: In vitro experiments conducted using three life-forms (adult, microfilariae and infective larva) of Acanthocheilonema viteae using different antifilarial agents. Study indicated that this in vitro screening system can be used for primary screening of potential antifilarial agents provided three life forms of A. viteae are used simultaneously to avoid false negative results.

In vivo models:

(1) Litomosoides carinu – cotton rat system: Mites (Ornithonyssus bacoti) allowed to feed on Litomosoides infected cotton rats (1 week) Then on clean cotton rats 2 weeks (development in mites takes 2 wks.) De-mited – microfilareae in blood after 50 days Rats with at least 250 microfilareae/mm3 blood used Drug in 1/5 MTD given i.p. for 6 days Blood examined for microfilareae weekly Rats showing disappearance of microfilareae after 7 wks autopsied Absence of adult worm in pleural & peritoneal cavities observed.

(2) Setaria cervi – rat system: Adult Setaria cervi collected from freshly slaughtered cattle.

Rats (100- 150 gms), anaesthetized with ether, incision ½" long in abdominal wall 2 male & 2 female worms put in peritoneal cavity Peritoneum, body wall stitched, Antiseptic applied daily Microfilareae in blood after 10±3 days, last for 54± 6 days Rats showing Microfilareae for 3 consecutive days used Treated with test drug orally/ i.p. Complete disappearance of Microfilareae from blood for 3 consecutive days – evidence of antifilarial action.

(3) Brugia pahangi in Mongolian jirds (Meriones unguiculatus): Mongolian Jirds < 1 yr. used. s.c. inoculation of 100 infective larvae of B. pahangi obtained from infected mosquitoes. Animals sacrificed at 90,140,200 days post infection Total adult worm recovery Location of worms—heart, lymphatics Microfilareae in peripheral blood

(4) Brugia malayi in Jirds: jirds infected subcutaneously with infective stage larvae (L3) of Brugia malayi evaluated as animal model for assessing macrofilaricides. Animals treated with a test compound. Change in microfilaria density observed. DEC at 50 mg/kg for 5 consecutive days for clearing the existing mf from the blood stream.

(5) Molinema dessetae in Proechimys oris: Evaluated drugs for antifilarial activity against Molinema dessetae in vivo in its natural host, the rodent Proechimys oris

(6) Wuchereria kalimantani in leaf monkeys : Trials in leaf monkeys (Presbytis cristatus) infected with Wuchereria kalimantani. Filaricidal drugs in given for 5 consecutive days to leaf monkeys (Presbytis cristatus) infected with Wuchereria kalimantani. Optimal microfilaricidal effect occurred at 200-mg/kg body weight of Ivermectin.

(7) Setaria digitata microfilaraemia in Mastomys coucha: an animal model for chemotherapeutic and immunobiological studies. Intraperitoneal implantation of adult gravid females of the bovine filarial parasite, Setaria digitata in Mastomys coucha (multimammate mice) was found to induce microfilaraemia lasting for about 125 days. Microfilariae could be detected 4 days post-implantation Peak levels (about 30 mf /20 µl blood observed by 21 days. The mf in circulation eliminated by oral adm. of DEC indicating usefulness of the model for screening potential anti-microfilarial drugs. Induction of antibodies to various fractionated antigenic components of adult parasites demonstrated by enzyme immunoassay in M. coucha implanted with live or cold-stunned adult worms. S. digitata-M. coucha model thus found amenable to perform chemotherapeutic and immunobiological investigations in experimental filariasis.

8) O. volvulus in chimpanzees Primate model for onchocerciasis research: Infected 18 chimpanzees by s.c. inj. of 250 third-stage larvae of O. volvulus. Six received test drug on day 1, another six received test drug on day 28 after infection, and six received no drug. Four control animals received no infective larvae and no drug. Developed a pattern of infection that closely resembles that seen in humans-formation of nodules by adult worms, the subcutaneous distribution of microfilariae Antibody responses have also been increasing with time.

ECHINOCOCCUS GRANULOSUS

(1) BALB/c mice infected with secondary equine E. granulosus : Echinococcus granulosus: the effects of praziquantel, in vivo and in vitro, on the ultrastructure of equine strain murine cysts. Richards KS, Morris DL, Daniels D, Riley EM.. Praziquantel (500 mg/kg) administered orally to BALB/c mice infected with secondary equine E. granulosus daily for 21, 30 or 30 + 30 days without the drug Ultrastructural examination of cysts showed

increased vesiculation of the germinal layer leading, in many, to the loss of its integrity. Increased mitochondrial numbers occurred frequently. Longer drug treatments appeared to have greater effects on germinal layer There was no detectable reestablishment of structural organization within 30 days after drug withdrawal. Tissue from collapsed cysts was necrotic. In an in vitro study at praziquantel concentrations of 1000 and 5000 micrograms/l over a 10-day period, most cysts showed ultrastructurally a time- and concentration-dependent loss of integrity identical to that seen in vivo.

(2) Echinococcus granulosus in Gerbils Morris DL, Taylor DH. (J Helminthol. 1990): Gerbils with well-developed peritoneal cysts of E. granulosus randomized to albendazole 50 mg/kg/day or untreated control.Treated animals had less disease at post mortem after 3 months of treatment Cysts were then taken from both albendazole-treated and control animals and cultured in vitro either with or without albendazole sulphoxide (Alb Sx) 500 micrograms/L for 14 days. Viability of cysts was then established by implantation of whole cysts into gerbils.

ASCARIS:

(1) Mouse-ascaris suum test model.

(2) Ascaris suum in experimentally infected pigs.

Persistent activity of doramectin and ivermectin against Ascaris suum in experimentally infected pigs. Study was conducted to investigate the persistent nematocidal activity of two avermectins against experimentally induced infections of Ascaris suum in swine.Seventy-two nematode-free cross-bred pigs of similar bodyweight were randomly allotted to nine treatment groups of eight pigs each.Eight of the groups were treated with injectable solutions containing 300 microg of doramectin/kg (IM) or 300 microg of ivermectin/kg (SC) either 0 (same day), 7, 14, or 21 days prior to an oral challenge of 50000 embryonated A. suum eggs.The ninth group (control) was challenged in parallel without any avermectin treatment.At 41 or 42 days after challenge, pigs euthanatized, adult & larval stages of A. suum were collected from the gastrointestinal tract of each pig and counted.

COLLECTION OF PLANT MATERIAL : Plant materials were collected from the Ayurvedic store. And were identified and authenticated at the department of Botany, osmania University, Hyderabad. The fresh barks properly washed in tap water and then shade dried. The dried bark is pulverized using mixer to obtain a powder form. These were stored in air tight containers protected from sunlight until required for analysis.

EXTRACTION

Maceration

In this process, the whole or coarsely powdered crude drug is placed in a volumetric flask with the solvent and allowed to boil for a period of at least 3 days with frequent agitation with reflux condenser until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

SUCCESSIVE SOLVENT EXTRACTION : Successive solvent extraction techniques were adopted to prepare various extract (petroleum ether, chloroform, alcohol) of *symplocus racemosa*.

SOXHELET EXTRACTION: The procedure is. In this method, the fi nely ground crude drug is placed in a porous bag or “thimble” made of strong fi lter paper, which is placed in chamber E ofthe Soxhlet apparatus The extracting

solvent in fl ask A is heated,and its vapors condense in condenser D. The condensed extractant dripsinto the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquidcontents of chamber E siphon into fl ask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently fi nancial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or largescale. The dried extracts obtained were stored in desiccators until they were used.

PROCEDURE:100gm of air dried powdered material of selected plants were successively extracted separately in soxhlet apparatus using petroleum ether, following by chloroform and methanol. Each time before extraction with the next solvent the powdered material was air-dried. Finally the marc obtained was macerated with methanol (40%) for 24 hours to get aqueous methanolic extract. All the extracts were then concentrated by distilling the solvent and evaporation them to dryness at low temperature. They were then weighed and the percentages of different extractive values were calculated in terms of air dried weight of plant material. The colour and consistency of the extracts were noted and results were tabulated.

QUALITATIVE CHEMICAL EXAMINATION : The extracts obtained from extraction were subjected to various chemical tests, for different constituents like, alkaloids, glycosides, carbohydrates, phenols and tannins, phytosterols, fixed oils and mucilage. The oil was analyzed by TLC.

PHYTOCHEMICAL TEST FOR EXTRACTS

Detection of carbohydrates: Small quantities of extracts were dissolved in 5ml of distilled water and filtered separated. The filtrates were used to test the present of carbohydrates.

a)Molish's test: Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

b)Benedict's test: Filtrates were treated with benedict's reagent and heated on water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.

c)Fehling's test: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of alkaloids : Small portions of extract were separately stirred with dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloidal reagents.

a)Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodine). Formation of an yellow cream precipitate indicates the presence o alkaloids.

b)Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide) and observed. Formation of brown / reddish brown precipitate indicates in presence of alkaloids.

c)Dragendroff's test: Filtrates were treated with Dragendroff's reagent (Solution of

potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

d)Hager's test: Filtrates were treated with Hager's reagent (Saturated picric acid solution). Formation of yellow coloured precipitate indicates the presence of alkaloids.

Detection of glycosides: Extracts were hydrolysed with dilute hydrochloric acid; the hydrolysis were subjected to glycoside test.

a)Modified Borntrager's test: The extract were treated with ferric chloride solution and immersed in boiling water bath for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. The formation of rose pink or cherry red colour in the ammonical layer indicates the presence of Anthranol glycosides.

b)Legal's test: The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to blood red colour indicates the presence of cardiac Glycosides.

Detection of saponins

a)Froth test: Dilute the extracts with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicates the presence of saponins.

b) Liberman Buchard's test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of brown ring at the junction indicates the presence of steroidal saponin's.

Detection of Phytosterols

a)Salkowski's test: The extracts were treated with chloroform and filtered separately. The filtrates were treated with few drops of concentrated sulphuric acid, shaken well and allowed to stand. The formation of a yellow coloured lower layer indicates the presence of free steroids.

b) Libermann- burchard's test: The extracts were treated with chloroform and filtered separately. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tubes. The formation of brown ring at the junction indicates the presence of phytosterols.

Detection of fixed oils and fats

a)Stain test: A small quantity of extracts was pressed between two filters papers separately. An oily stain on filter paper indicates the presence of fixed oil.

b)Soap test: The extracts were heated on water bath with 0.5N alcoholic potassium hydroxide solution. Formation of soap indicates the presence of fixed oils and fats.

Detection of Phenolics and Tannins

a)Ferric chloride test: Treat the extracts with few drops of neutral ferric chloride solution. The formation of Bluish black colour indicates the phenolic nucleus.

b)Gelatin test: To the extract added 1% gelatin solution containing sodium chloride. The formation of white precipitate indicates the presence of tannins.

c)Lead acetate test: The extracts were treated with lead acetate solution. Formation of yellow precipitate indicates the presence of flavanoids.

d)Alkaline reagent test: The extracts were treated with few drops of sodium hydroxide

separately. Formation of Intense yellow colour, which becomes colourless on addition of few drops of dilute acid, indicates the presence of flavanoids.

e)Vanillin hydrochloride test: The extracts were treated with few drops of vanillin hydrochloride reagent. The formation of pinkish red colour indicates the presence of tannins.

f) Shinoda test: The extracts were treated with few fragments of magnesium metal separately, followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta colour indicates the presence of flavonoids.

Detection of proteins and amino acids

a)Millon's test: The extracts were treated with 2 ml of million's reagent. The formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

b)Biuret test: The extracts were treated with 1 ml of 10% sodium hydroxide solutions and heated. Add a drop of 0.7% copper sulphate solution to the above mixtures. The formation of purplish violet colour indicates the presence of proteins.

c)Ninhydrin test: To the extracts added 0.25% ninhydrin reagent and boiled for few minutes. Formation of blue colour indicates presence of amino acid.

THIN LAYER CHROMATOGRAPHY: Thin layer chromatography is performed by taking the suitable solvent system just to check the presence of ellagic acid

HPTLC[4] :Thin layer chromatography (TLC); also know as planar-chromatography or flat bed chromatography is like all other chromatographic techniques, a multi-stage distribution process.

HPTLC is the most simple separation technique today available to the analyst. It can be considered a time machine that can speed your work and allows you to do many things at a time usually not possible with other analytical techniques[23-25].

TLC / HPTLC are often found more troublesome than GLC / HPLC as quantitative TLC is an off-line technique, hence automation is difficult and because of its open character, is highly influenced by environmental factors. It is, therefore, essential that each step which may require specific approach must be carefully validated to determine potential source of error.

Factors influencing the TLC / HPTLC separation and resolution of spots: Type of stationary phase(sorbent),Type of pre-coated plates(TLC / HPTLC);for quantitative analysis, use of HPTLC precoated, is absolutely essential, Layer thickness / Binder in the layer, Mobile phase(solvent system),Solvent purity, Size of the developing chamber, Saturation of chamber(pre-equilibrium),Sample volume to be spotted ,Size(diameter) of the initial spot,Solvent level in the chamber, Gradient, Relative humidity, Temperature(*RF* values usually increase with rise in temperature), Flow rate of solvent, Separation distance, Mode of development.

Greater the distance between different spots and smaller the initial spot diameter of the sample, better the resolution.

While describing the result of any TLC / HPTLC procedure, various parameters and conditions under which results for a specific analysis have been obtained must be documented. This is absolutely essential for possible results.

Features of HPTLC : 1. Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal

Standard. 2. Several analysts work simultaneously.3. Lower analysis time and less cost per analysis.4. Low maintenance cost.5. Simple sample preparation - handle samples of divergent nature.6. No prior treatment for solvents like filtration and degassing.

7. Low mobile phase consumption per sample.8. No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination.9. Visual detection possible - open system. 10.Non UV absorbing compounds detected by post-chromatographic derivatization.

Steps involved in HPTLC: 1. Selection of chromatographic layer.2. Sample and standard preparation.3. Layer pre-washing. 4. Layer pre-conditioning .5. Application of sample and standard.6. Chromatographic development .7. Detection of spots.8. Scanning.9. Documentation of chromatic plate

Selection of chromatographic layer: Precoated plates-different support material-different sorbents available, 80 % of analysis : Basic substances, alkaloids and steroids, Aluminum oxide- silica gelGF, Amino acids, dipeptidees, sugars and alkaloids- cellulose, Non-polar substances, fatty acids, caratenoids, cholesterol-RP-2,RP-8 and RP-18, Preservatives, barbiturates, analgesic and phenothiazines-Hybrid plates- RP-WF254s.

Sample and standard preparation: To avoid interference from impurities and vapours, Low signal to noise ratio-straight base line-Improvement of LOD, Solvents used are Methanol, Chloroform : Methanol (1:1), Ethyl acetate : Methanol (1:1), Chloroform : Methanol : ammonia (90:10:1), Methylene chloride : Methanol (1:1), 1 % Ammonia or 1 % Acetic acid, Dry the plates and store in dust free atmosphere.

Activation of pre-coated plates: Freshly open box of plates do not require activation, By placing in an oven at 110-120o C for 30 minutes prior to spotting, Plates exposed to high humidity or kept on hand for long time to be activated, Aluminum sheets should be kept in between two glass plates and placing in oven at 110^0 C,120^0 C for 15 minutes.

Application of sample and standard: Usual concentration range is 0.1-1 µg / µl,, Above this cause poor separation, Linomat IV(automatic applicator) – nitrogen gas sprays sample and standard form ,syringe on TLC plates as bands, Band wise application-better separation-high response to densitometer.

Selection of mobile phase: Trial and error, One's own experience and Literature, 3-4 component mobile phase should be avoided, Multi component mobile phase once used not recommended for further use and solvent , composition is expressed by volume (v / v) and sum of volumes is usually 100, Twin trough chambers are used only 10-15 ml of mobile phase is required, Components of mobile phase should be mixed introduced into the twin-trough chamber.

Normal phase : Stationary phase is polar, Mobile phase is non polar, Non-polar compounds eluted first because of lower affinity with stationary phase, Polar compounds retained because of higher affinity with the stationary phase.

Reversed phase : Stationary phase is non polar, Mobile phase is polar, Polar compounds eluted first because of lower affinity with stationary phase, Non-Polar compounds retained because of higher affinity with the stationary phase.

Pre- conditioning (Chamber saturation): Un-saturated chamber causes high *RF* values, Saturated chamber by lining with filter paper for 30 minutes prior to development uniform , distribution of solvent vapours- less solvent for the sample to travel- lower *RF* values.

Chromatographic development and drying: After development, the plate and mobile phase is removed from the plate- to avoid contamition of lab atmosphere, Dry in vacuum desicator (avoid hair drier) because essential oil components may evaporate.

Detection and visualization: Detection under UV light is first choice- non destructive, Spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length), Spots of non fluorescent compounds like ethambutol, dicylomine etc- dipping the plates in % iodine solution, When individual component does not respond to UV- derivatisation required for detection.

Quantification: Sample and standard should be chromatographed on same plate-after development chromatogram is scanned, Camag TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode-scanning speed selectable up to 100 mm / s – spectra recording is fast-36 tracks with up to 100 peak windows can be evaluated, Calibration of single and multiple levels with linear or non-linear regressions are possible when target values are to be verified such as stability testing and dissolution profile, single level calibration is suitable, Statistics such as RSD or CV report automatically, Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

Documentation: E-Merck introduced plates with imprinted identification code, supplier name, item number, batch number and individual plate number, Avoid manipulation of data at any stage, All work performed is documentated in a project worksheet.

Parameters that are affected by the changes in chromatographic conditions are:

· Retention factor (*RF*),

· Peak purity.

1. Retention factor (*RF*): Retention factor (*RF*) is defined as the amount of separation due to the solvent migration through the sorbent layer as shown in the formula. It depends on time of development and velocity coefficient or solvent front velocity.

$$RF = \frac{\text{Migration distance of substance}}{\text{Migration distance of solvent front from origin}}$$

purity: The null hypothesis "these spectra are identical" can in this case (purity) with two sided significance. During the purity test the spectrum taken at the first peak slope is correlated with the spectrum of peak maximum [r (s, m)] and the correlation of the spectra taken at the peak maximum with the one from the down slope or peak end [r (m, e)] which is used as a reference spectra for statistical calculation. An error probability of 1 % only be rejected if the test value is greater than or equal to 2.576.

HPTLC Method design and development: Set the analytical objective first that may be quantification or qualitative identification or separation of two components/multicomponent mixtures or optimization of analysis time before starting HPTLC. Method for analyzing drugs in multicomponent dosage forms by HPTLC demands primary knowledge about the nature of the sample, namely, structure, polarity, volatility, stability and the solubility parameter. An exact recipe for HPTLC, however, also same like HPLC cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, with what kind of mobile phase.

Selection of stationary phase is quite easy that is to start with silica gel which is

reasonable and nearly suits all kind of drugs. Mobile phase optimization is carried out by using three level techniques. First level involves use of neat solvents and then by finding some such solvents which can have average separation power for the desired drugs. Second level involves decreasing or increasing solvent strength using hexane or water for respective purposes. Third level involves trying of mixtures instead of neat solvents from the selected solvents of first and second level which can further be optimized by the use of modifier like acids or bases. Analytes are detected using fluorescence mode or absorbance mode. But if the analytes are not detected perfectly than it need change of stationary phase or mobile phase or need the help of pre or post chromatographic derivatization. Optimization can be started only after a reasonable chromatogram which can be done by slightchange in mobile phase composition. This leads to a reasonable chromatogram which has all the desired peaks in symmetry and well separated.

EVALUATION OF ANTHELMINTICS ACTIVITY[5]: Helminthiasis, commonly known as worm infection is one of the most prevalent disease and cause most seriousness to public health .It is prevalent globally (1/3 of the world's population harbours them), but it is most developing countries with poorer personal and environmental hygiene. In the human body, gait. is the abode of harm helminthes ,but some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving him by food, causing blood loss, injury to organs, intestinal or lymphatic obstruction or by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of ill in health. Helminthes infect human host are divided into two categories. These areas- Aschelminthes or Nematodes (round worms) e.g. hook worm (Necator ammericana), pin worm (Enterobiasis vermiculasis), round worm (Ascariasis lumbricoides), whipworm (Trichuris tricura). Trematodes (flukes or schitosomes) e.g. beef tapeworm (Taenia saginata),pork tapeworm(Taenia solium) [27-31]

Availability of drug therapy:"Anthelmintic are drugs that either kill (vermicide) or expel (vermifuge) infesting helminthes." These are act either locally to expel worm from the gastrointestinal tract or systematically to eradicate adult helminths .To control or treat helminthes infection various of compounds are present depending upon the parasites. The drugs that are being used in helminthes infections are classified into following groups.

Benzimidazole : These are broad spectrum anthelmintic discovered in 1960's with a high activity against the gastrointestinal helminthes. Thousands of Benzimidazole derivatives were screened for anthelmintic activity and their development and chemistry reviewed by Townsend and Wise .From these derivatives only Mebendazole ,Albendazole and Thiobendazole now marked existence.

Diethylcarbamazine: Developed in 1940's and proven to be especially effective as a filaricidal agent. It is chemically similar to Piperazine but it is active against filarial and microfilaria while piperazine is only active against nematodes

Diethylcarbamazine: (DEC) is an anthelmintic drug that does not resemble other antiparasitic compounds. It is a synthetic organic compound which is highly specific for several parasites and does not contain any toxic metallic elements.

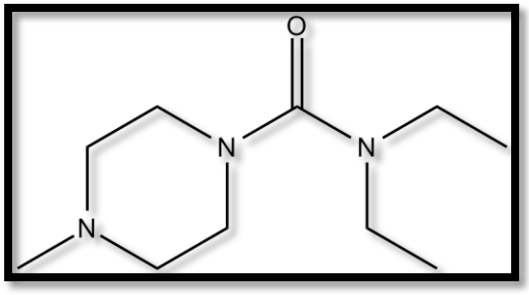


FIG: STRUCTURE OF DIETHYLCARBAMAZINE

Uses : DEC is indicated for treatment of individual patients with certain filarial diseases. These diseases include: lymphatic filariasis caused by infection with *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori*; tropical pulmonary eosinophilia, and loiasis. In cases of onchocerciasis, another common filarial parasite, the drug is not used. This is because of the intense and unbearable itching associated with the dead subcutaneous parasites.DEC continues to be the mainstay for treatment of patients with lymphatic filariasis and loiasis. DEC is also used in the prevention of dog heartworm *Dirofilaria immitis*.

Tapeworms/cestodes

It is also used to treat: Echinococcosis , Cysticercosis (though it has been judged less effective than albendazole in treatment of neurocysticercosis.), Intestinal tapeworms. In veterinary medicine it is widely used against tapeworms, either alone under the trade name Droncit, or in combination with pyrantel pamoate under the trade name Drontal.

Chemical data : Formula $C_{10}H_{21}N_3O$, Mol. mass 199.293 g/mol , SMILES eMolecules & PubChem

Mechanism: DEC is an inhibitor of arachidonic acid metabolism in filarial microfilaria. This makes the microfilaria more susceptible to immune attack.

Trade names: Hetrazan, Carbilazine, Caricide, Cypip, Ethodryl, Notézine, Spatonin, Filaribits, Banocide Forte

Ivermecttine: Extracted from the soil actinomycets, the natural Ivermectine,which are 16 members macroyclic lactones upon redection of the C22 – C 23 double bond give rise to Ivermectine. Ivermectine has proven to be quite beneficial for the treatment of various nematode infections.

Praziquantel: Praziquantel is an isoquiniline derivative,which is most effective cestode and nematode infections. Praziquantel (Biltricide) is an anthelmintic effective against flatworms. Praziquantel is not licensed for use in humans in the UK; it is, however, available as a veterinary anthelminthic, and is available for use in humans on a named-patient basis.

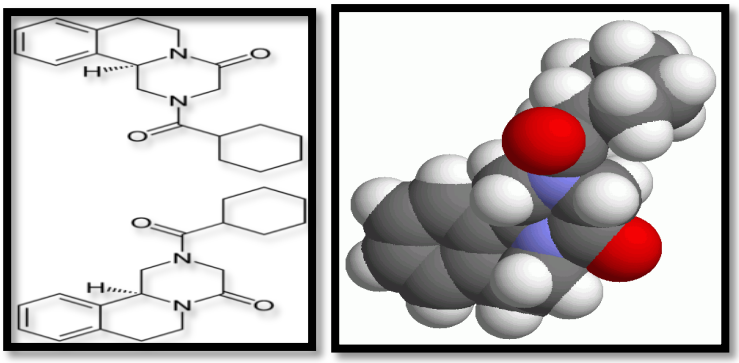


FIG: STRUCTURE OF PRAZIQUANTEL

Chemical data: Formula $C_{19}H_{24}N_2O_2$, Mol. mass 312.411

Pharmacokinetic data :Bioavailability - relatively small, Metabolism -hepatic ,Half life -0.8 to 1.5 hours (Main Metabolites 4 to 5 hours) ,Excretion- mainly in urine,Therapeutic considerations:

Pregnancy cat: Only when clearly needed (lack of sufficient data in humans),Legal status -U.S.: Rx-only (human use), over-the-counter (veterinary use), Routes- oral

Uses : It is used against *Schistosoma*. As of 2005, praziquantel is the primary treatment for human schistosomiasis, for which it is usually effective in a single dose. It is used to treat liver flukes (such as *Clonorchis sinensis*) except for fascioliasis. It is also used to treat paragonimiasis.

History: Praziquantel was developed in the laboratories for parasitological research of Bayer AG in Germany (Elberfeld) in the mid 1970s. The World Health Organization includes it on its Model List of Essential Medicines.

Pharmacokinetics: Praziquantel is well (approximately 80%) absorbed from the gastrointestinal tract. Due to extensive first-pass metabolization only relatively small amounts enter systemic circulation. Praziquantel has a serum halflife of 0.8 to 1.5 hours (metabolites 4 to 5 hours) in adults with normal renal and liver function. In patients with significantly impaired liver function (Child Pugh classes B and C) the serum halflife is increased to 3 to 8 hours. Praziquantel and its metabolites are mainly excreted in the urine, and within 24 hours after a single oral dose, 70 to 80% are found in urine, but less than 0.1% are found as the unchanged drug. Praziquantel is metabolized through the cytochrome P450 pathway 3A4. Agents that induce or inhibit Cyp450 3A4 (ie phenytoin, rifampin, azole antifungals) will have an effect the metabolism of praziquantel.

Praziquantel has a particularly dramatic effect on patients with schistosomiasis. Studies of those treated have shown that within six months of receiving a dose of praziquantel, up to 90% of the damage done to internal organs due to schistosomiasis infection can be reversed.

Mode of action : Although the mode of action is not exactly known at present, there is experimental evidence that Praziquantel increases the permeability of the membranes of parasite cells (certain schistosomes) for calcium ions. The drug thereby induces contraction of the parasites resulting in paralysis in the contracted state. The dying parasites are dislodged from their site of action in the host organism and may enter systemic circulation or may be destroyed by host immune reaction (phagocytosis).

Additional mechanisms — focal disintegrations and disturbances of oviposition (laying of eggs) — are seen in other types of sensitive parasites.Another hypothesis on the mechanism of action of praziquantel has been recently reported. The drug seems to interfere with adenosine uptake in cultured worms. This effect may have therapeutical relevance given that the schistosome, as the taenia and the echinococcus (other praziquantel sensitive parasites), is unable to synthesize purines *de novo*.

Side effects : The majority of side-effects develop due to the release of the contents of the parasites as they are killed and the consequent host immune reaction. The heavier the parasite burden, the heavier and more frequent the side effects normally are.

- Central nervous system: Frequently occurring side effects are dizziness, headache, and malaise. Drowsiness, somnolence, fatigue, and vertigo have also been seen. Almost all patients with cerebral cysticercosis experience CNS side effects related to the cell-death of the parasites (headache, worsening of preexisting neurological problems, seizures, arachnoiditis, and meningism). These side effects may be life-threatening and can be reduced by coadministration of corticosteroids. It is strongly recommended that all patients with cerebral cysticercosis are hospitalized during treatment.

- GI Tract: Approximately 90% of all patients have abdominal pain or cramps with or without nausea and vomiting. Diarrhea may develop and may be severe with colic. Sweating, fever, and sometimes bloody stools may occur together with diarrhea.

- Liver: Asymptomatic and transient increases of liver enzymes (AST and ALT) are noted frequently (up to 27%). No case of symptomatic liver damage has ever been seen so far.

- Sensitivity reactions: Urticaria, rash, pruritus and eosinophilia in White Blood Counts.

- Other locations/Body as a whole: Lower back pain, myalgia, arthralgia, fever, sweating, various cardiac arrhythmias, and hypotension.

Drug interactions

Antibiotics: A study found that the Antibiotic rifampicin decreases plasmatic concentrations of praziquantel.

Antiepileptics : Carbamazepine and phenytoin are reported to reduce the bioavailability of praziquantel.

Antimalarials : Chloroquine reduces the bioavailability of praziquantel.

Antacids / histamine H_2-antagonists : At least 2 studies indicate the drug Cimetidine heightens praziquantel bioavailability.

Dosage : According to special dosing schedules for each different indication. Sometimes one single dose or a one-day treatment with divided doses may be sufficient.

Brand names: Biltricide (Bayer) 600 mg Tablets (for human use), Cesol (Merck) Tablets, Cysticide (Merck) Tablets , Zentozide (Berich (Thailand) Co), Profender (combination with emodepside) (Bayer) for veterinary use , Droncit (Bayer) for veterinary use , Drontal (combination with pyrantel pamoate) (Bayer) for veterinary use , D-Worm (Farnum) for veterinary use; note that D-Worm also makes roundworm medicine containing piperidine which is not effective against tapeworms , Tape

Worm Tabs (Trade Winds) for veterinary use , Cestoved (Vedco) both tablets and injectable for veterinary use , PraziPro (Hikari) for aquarium use.

Pyrantelpalmoate: Pyrantelpalmoate was first reported for its anthelmintic activity in 1966. It is considered as a drug of choice for the treatment of pinworms. In addition ,it also effective in hookworm and round worm infections.

Pyrantel pamoate (under US Pharmacopoeia) or Pyrantel Embonate (under European Pharmacopoeia), is used as a deworming agent in the treatment of hookworms (all species)

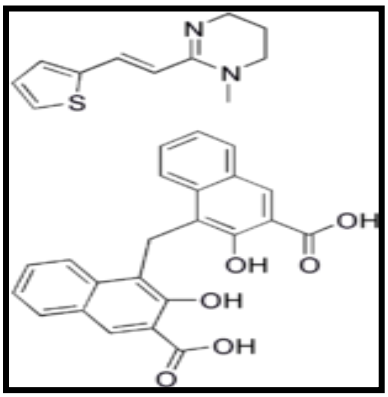


FIG: STRUCTURE OF PYRANTELPALMOATE

Identifiers CAS number [22204-24-6] ,PubChem 5281033 ,MeSH Pyrantel+pamoate

Properties : Molecular formula $C_{34}H_{30}N_2O_6S$, Molar mass 594.6768

Drug action : Pyrantel pamoate acts as a depolarizing neuromuscular blocking agent, thereby causing sudden contraction, followed by paralysis, of the helminths. This has the result of causing the worm to "lose its grip" on the intestinal wall and be passed out of the system by natural process. Since Pyrantel is poorly absorbed by the hosts intestine, the small dosage of medication used is completely ineffective to the host. Spastic (tetanic) paralyzing agents, in particular pyrantel pamoate, may induce complete intestinal obstruction in a heavy worm load. This obstruction is usually in the form of a worm impaction and happens when a very small, but heavily parasitized animal is treated and tries to pass a large number of dislodged worms at once. Worms usually pass in normal stool or with diarrhea, straining, and occasional vomiting. Dosage is 11 mg/kg not to exceed 1 g as a single dose.

and roundworms (*Parascaris equorum*, aka ascarids in humans) in domesticated animals such as horses, cattle, sheep, pigs, cats, dogs, and many other species. It is a combination of pyrantel and pamoic acid. Some drug companies pair pyrantel pamoate with praziquantel for tapeworms, and sometimes febantel for whipworms in order to provide more complete treatment for intestinal parasites in one dose. It is also used for pinworm treatment for humans in many brands, including "Reese's Pinworm Medicine", "Pin-X", "Pin-Rid", "Combantrin", "Anthel", "Helmintox", "Helmex".Pyrantel pamoate is also commonly included in monthly administered chewable heartworm tablets for cats and dogs.

Toxicity : Pyrantel pamoate is considered a class C drug for use during pregnancy for humans, but is a class A for canines and felines. Pyrantel is considered safe to use in nursing animals.

Choice of drug for Helminthiasis:

Worms	First choice of drugs	Alternative
1.Round worm Ascaris lumbricocids	Mebendazole, Albendazole, Pyrentel	Piperazine,Levamisole Ivermectine,Pyrentel
2. Hook worm Necator americanus	Mebendazole, Albendazole	Piperazine
3. Thread worm Enterobius vermicularis	Pyrentel, Mebendazole, Albendazole	Albendazole, Thiobendazole
4. Strongyloides stercorali	Ivermectin	Albendazole
5. Wipe worm	Mebendazole ,Albendazole	Mebendazole
Trichuruis trichiura 6. Trichinella spiralis	Diethyl carbamazine	Albendazoze
7. Filaria Wuchereria bancrofti	Metronidazole	Thiobendazole
8. Guinea worm Dracunculus medimensis	Praziqantel	Albendazole
9. Tape worms Taenia saginata Taenia solium Hymenopepis nana Neurocysticercosis	Praziquantel, Praziquantel, Praziquantel, Albendazole	Albendazole Niclosamide Niclosamide Praziquantel
10. Hydatid disease	Albendazole	Mebendazole

METHOD AND MATERIALS:

Plant and plant materials : Barks of *Symplocos racemosa* were collected, authenticated, shade dried and extracted with petroleum ether, chloroform and ethanol by successive extraction method using Soxhlet apparatus. The solvent was seprated from the crude extract by reduced pressre evaporator. .The concentrated crude extract were subjected to phytochemical screening, the powdered plant material was extracted successively with petroleum ether (60-80^{0}C) , chloroform and ethanol using Soxhlet apparatus .The solvents were then removed under reduced pressure, which obtained sticky residues.

Drug Used : Piperazine citrate and albendazole were used as reference standards .

Anthelmintic Activity : The `anthelmintic activity was evaluated on adult Indian earthworm, Pheretima postthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasities of human beings.The method of Ghos et al. was followed for this study .Eighteen groups of approximately equal sized Indian earthworm consisting of six earthworms in each group were released in to 50ml of desired formulation .

Each group was treated with one of the following : vehicle (1% gum acacia in normal saline), piperazine citrate (15mg/ml) ,albendazole (10mg/ml) or extracts (2.5,5,10, 25 or 50 mg/ml) .Observations were made for the time taken to paralyse and/or death of individual worms .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 away of their body colour.

RESULTS : In the anthelmintic study it was observed that the ethanolic extract was more potent than the other two extracts (petroleum ether and chloroform) even through all the three extracts were endwed with anthelmintic property. The order of activity was Ethanol extact>Chloroform extract>Pet-ether extract. The activity revealed concentration dependence nature of the different extracts .Potency of the extracts was found to be inversely proportional to the time taken for taken for paralysis/death of the worms.Table No.1-3

Anthelmintic Activity : The earthworm's *P. posthuma* were collected from moist soil and washed with normal saline to remove all the faecal matter. The anthelmintic activity was evaluated on adult Indian earthworms, *P. posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.10, 11 Six groups of earthworms were made consisting six in each group were released into 10 ml 5% DMF in normal saline of desired extract and standard drugs in petri dish at room temperature. Each group was treated with vehicle (5% DMF in normal saline) or albendazole (20 mg/ml) or ethanolic extracts of barks *Symplocos racemosa* (20 mg/ml, each). The time taken by worms to paralysis and death was noted. Death was ascertained by applying external stimuli, which stimulate and induce movements in worms as well as fed of the body color was noted.

In second part of experiment six groups of earthworms were made consisting six in each group were released into 10 ml 5% DMF in normal saline of desired extract and standard drugs in petri dish at room temperature. Each group was treated with vehicle (5% DMF in normal saline) or Albendazole or petroleum ether extract or chloroform extract or

ethanolic extract (20 mg/ml, each). The time taken by worms to paralysis and death was noted. Death was ascertained by applying external stimuli, which stimulate and induce movements in worms as well as fed of the body color was noted.

Table 1: colour, appearance, and yield of different extracts

S. no.	Different solvents	Colour of extracts	Appearance	Yield (%)
1	Petroleum ether extract	Green	Sticky	**3.4**
2	Chloroform extract	Reddish-brown	Sticky	**2.1**
3	Ethanol extract	Black	Dry	**12.5**

Table 2: phytochemical screening of different extracts

		Preliminary phytochemical test of phytoconstituents		
S. no.	**Tests**	**Pet. Ether**	**Chloroform**	**Ethanol**
1	**Carbohydrate**			
a)	*Benedict's test*	Negative	Negative	Positive
b)	*Molisch's test*	Negative	Negative	Positive
c)	*Fehling's test*	Negative	Negative	Positive
d)	*Barfoed's test*	Negative	Negative	Positive
2	**Proteins**			
a)	*Xanthoproteic test*	Negative	Negative	Negative
b)	*Million's test*	Negative	Negative	Negative
c)	*Biuret test*	Negative	Negative	Negative
d)	*Ninhydrin test*	Negative	Negative	Negative
3	**Sterols**			
a)	*Salkowaski test*	Positive	Positive	Negative
b)	*Liebermann Burchadt test*	Positive	Positive	Negative

c)	*Sulpher test*	Positive	Positive	Negative
4	**Triterpenoids**			
a)	*Salkowaski test*	Positive	Positive	Negative
b)	*Liebermann Burchardt test*	Positive	Positive	Negative
5	**Glycosides**			
a)	*Keller Killiani test*	Negative	Negative	Positive
b)	*Legal's test*	Negative	Negative	Positive
c)	*Guignard test*	Negative	Negative	Positive
d)	*Kedde's test*	Negative	Negative	Positive
6	**Saponins**			
c)	*Foam test*	Negative	Negative	Negative
e)	*Haemolysis test*	Negative	Negative	Negative
7	**Alkaloids**			
a)	*Mayer's test*	Negative	Positive	Positive
b)	*Hager's test*	Negative	Positive	Positive
c)	*Dragendorff's test*	Negative	Positive	Positive
d)	*Wagner's test*	Negative	Positive	Positive
8	**Flavonoids**			
a)	*Zinc–HCL-reduction test*	Negative	Negative	Positive
c)	*Lead acetate solution test*	Negative	Negative	Positive
d)	*Ferric chloride test*	Negative	Negative	Positive
e)	*Shinoda test*	Negative	Negative	Positive
9	**Tannins**			
a)	*Gelatin test*	Negative	Negative	Positive
b)	*Ferric-chloride test*	Negative	Negative	Positive

ESTIMATION OF ELLAGIC ACID IN LODHRA BARK BY HPTLC ANALYSIS

Figure1: Densitogram showing of ellagic acid extract using hexane: ethylacetate (8:2 v/v) at 254 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area AU	Area %
1	0.09 Rf	0.6AU	0.11Rf	27.9 AU	8.20%	0.12Rf	5.4AU	322.5	03.49%
2	0.12Rf	06.1AU	0.13Rf	34.1AU	10.04%	0.16Rf	0.3AU	474.4	05.13%
3	0.20 Rf	00.6 AU	0.24 Rf	47.7 AU	14.02%	0.26 Rf	19.7AU	1053	11.40%
4	0.26 Rf	20.3 AU	0.27 Rf	22.1 AU	06.49%	0.28 Rf	11.3 AU	312.0	03.38%
5	0.36 Rf	24.6 AU	0.44 Rf	169.5 AU	49.84%	0.47 Rf	2.3 AU	5932.7	64.21%
6	0.51 Rf	0.10 AU	0.54 Rf	16.2 AU	4.76%	0.57 Rf	0.3 AU	387.2	04.19%
7	0.59 Rf	3.50 AU	0.64 Rf	22.6 AU	6.66%	0.68 Rf	0.2 AU	757.1	08.19%

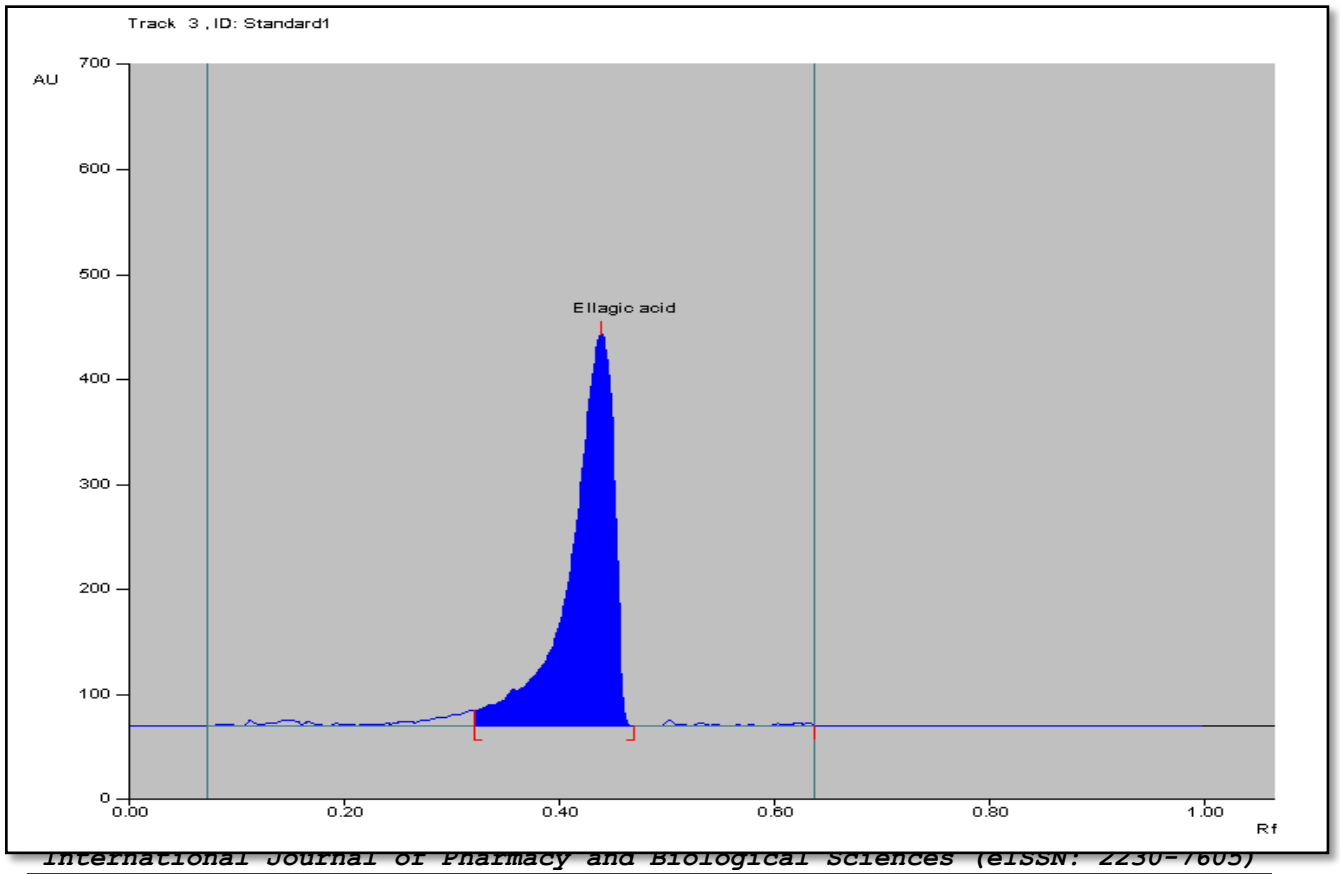


Figure 2: Densitogram showing of ellagic acid standard using hexane: ethylacetate (8:2 v/v) at 254 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area AU	Area %
1	0.32 Rf	14.2 AU	0.44 Rf	372.9 AU	100%	0.47 Rf	0.0 AU	12871	100%

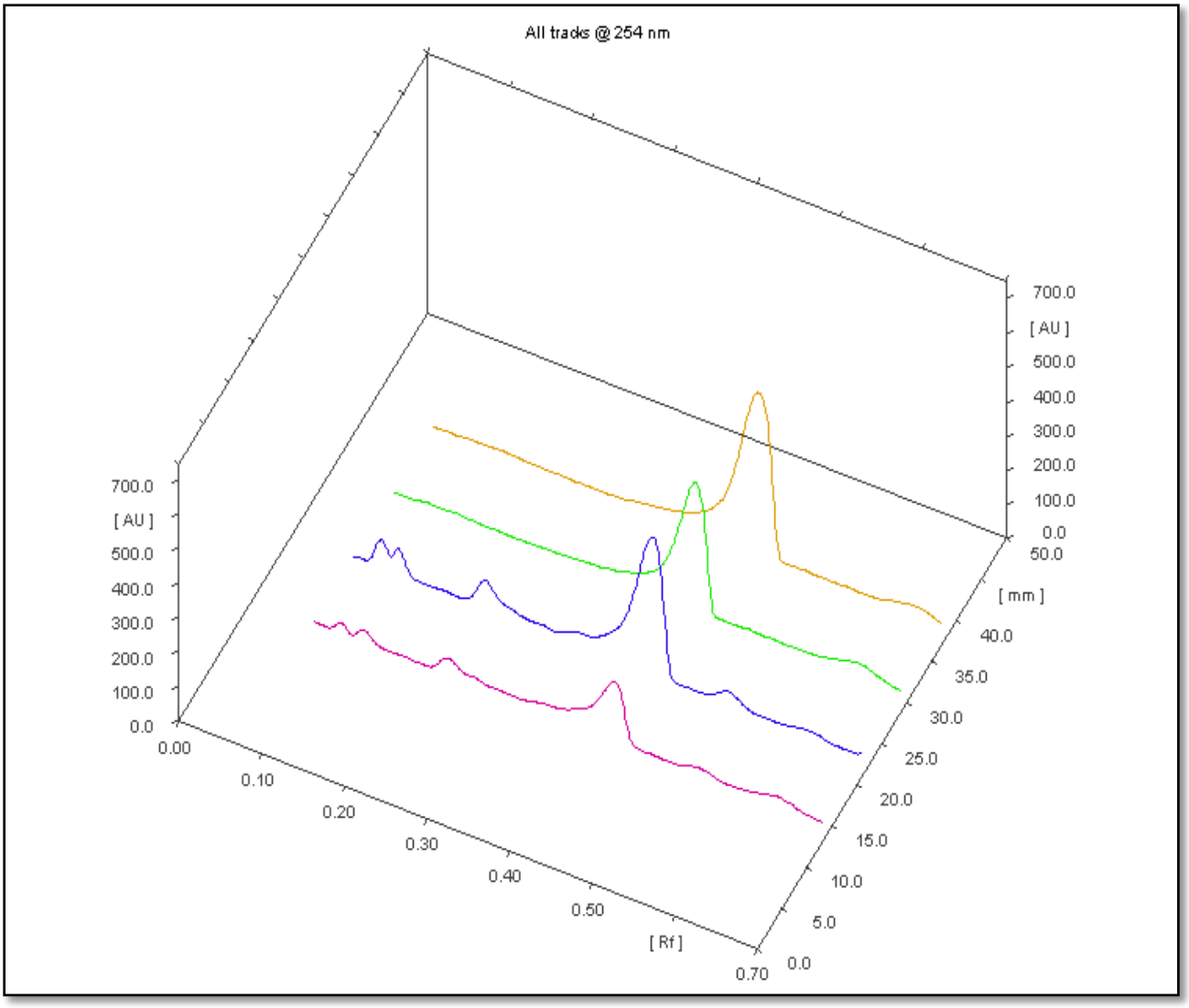


Figure 3.Three dimensional densitogram of ellagic acid in extract (1, 2 tracks) and standard (3, 4 tracks).

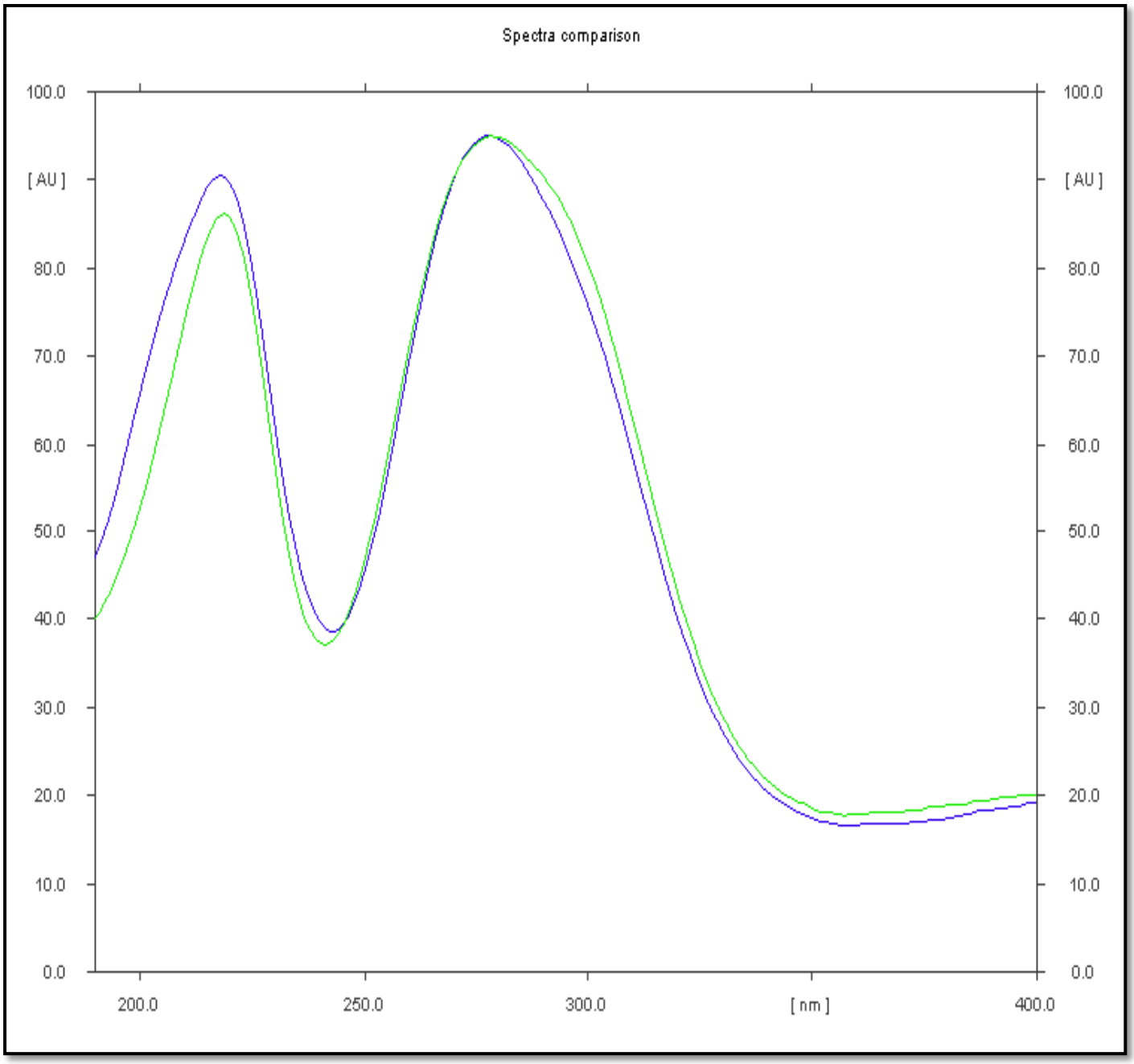
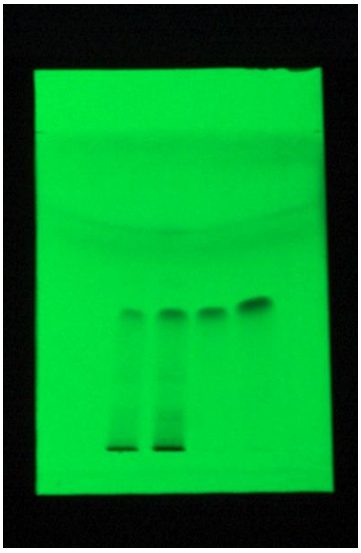
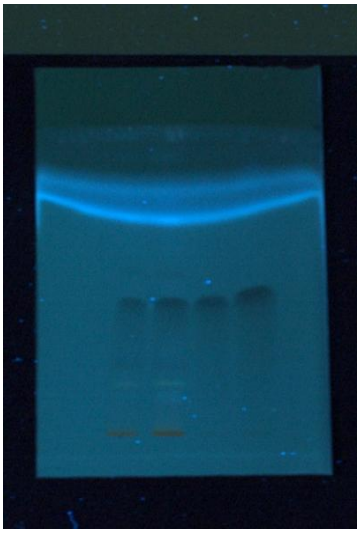


Figure 4: In situ UV spectra of extract (blue)and standard (green) by HPTLC



EX EX ST ST

Figure 5: Video image at 254nm.



EX EX ST St

Figure 6: Video image at 366nm.

Table 3: Anthelmintic activity of bark extract of *SYMPLOCOS RECEMOSA.*

Group	Standard/*Symplocos racemosa extract*	Concentration mg/ml	time taken for paralysis(min)	time taken for death (min)
1	VEHICLE	-	-	-
2	PIPERIZINE CITRATE	10	18.50±0.31	-
3	ALBENDAZOLE	15	34.66±0.72	63.83±0.79
4	PET ETHER	2.5	37.815±0.81	-
5	PET ETHER	5	24.841±1.85	33.08±3.098
6	PET ETHER	10	17.39±0.539	28.543±1.065
7	PET ETHER	25	16.788±0.378	21.441±1.100
8	ETHANOL	50	8.877±0.310	18.11±1.67
9	ETHANOL	2.5	26.79±1.33	-
10	ETHANOL	5	18.83±0.805	25.84±0.776
11	ETHANOL	10	15.56±1.752	20.57±0.6879
12	ETHANOL	25	13.716±0.596	19.23±0.855

13	CHLOROFORM	50	7.42±0.589	14.588±0.2733
14	CHLOROFORM	2.5	27.683±0.811	-
15	CHLOROFORM	5	21.433±0.854	27.783±0.331
16	CHLOROFORM	10	17.65±1.075	25.866±0.388
17	CHLOROFORM	25	16.58±0.304	20.5±1.165
18	CHLOROFORM	50	8.11±0.0813	14.9±0.199

SUMMARY AND CONCLUSION: Lodhra *(Symplocos racemosa)* bark successive extract was screened for its phytochemical and anthelmintic activity. Preliminary phytochemical activity revealed the presence of alkaloids, steroids, terpenoids, flavanoids and tannins.

ANTHELMINTIC ACTIVITY: The present investigation reveals that the ethanolic extract was endowed with potent anthelmintic property as compare to other extract. The pet. ether and chloroform extracts were also possess significant activity. The activity reveals concentration dependence nature of different extracts. the extract were also found to be lethal to warms up to concentration of 10mg /ml in pet. ether extract and up to 5mg /ml in other two and paralysis occurred upto a concentration of 5 mg/ml in all the extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis / death of the worms. The activities were comparable with the reference drug Piperazine citrate, Albendazole. The above consideration justify the enthelmintic properties of the plant as suggested by the folklore practice.

The practice of medicinal chemist is primary devoted to the discovery & development of new agent for treating the diseases. The use of plant and plant extracts for medicinal purpose is used for thousand years. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects.**Fig No.1-5**

HPTLC ANALYSIS : Preliminary phytochemical activity revealed the presence of tannins an attempt was carried out to standardize the extract by HPTLC analysis of estimation of ellagic acid in the ethanolic extract of lodhra. **Fig No.3**

CONCLUSION : Lodhra bark in the present study has showed potent anthelminitic activity which could be attributed to the presence of tannins, which was quantified with ellagic acid by HPTLC analysis.

BIBILOGRAPHY:

1. SULEIMAN, M. M., M. MAMMAN, Y. O. ALIU, J. O. AJANUSI: Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats. Vet. arhiv 75, 487-495, 2005.

2. Kritikar KR, Basu BD. Indian medicinal plants., Periodical Experts, New Delhi, Vol. I, 1935; pp-1511-13.

3. SINGHAL, K. C., SAXENA. P. N. and JOHRI. M. B. L. (1973): Studies on the use of Setaria cervi for in vitro antifilarial

screening, Jap. J. Pharmacology, 23, 793-797.

4. Sherma J, Fried B. Handbook of thin layer chromatography. 3rd ed. New York: MarcelDekker, Inc.; 2003. p. 3-4.

5. Ridtitid W, et al. Rifampin markedly decreases plasma concentrations of praziquantel in healthy volunteers. Clin Pharmacol Ther 2002; 72: 505–13.

6. Nadkarni AN. In, Indian Materia Medica, Vol. I: Popular Book depot, Bombay, India, 1989; pp-1186.

7. 7.Anonymous.TheAyurvedicPharmacopoeia of India, Part-1, Government of India, Ministry of Health and Family Welfare, Department of Health: New Delhi, India, 1978; pp- 82.

8. 8.Dhan R, Jain GK, Sarin PS and Khanna NM. Indian J Chem., 28B, 1989;982-986.

9. 9.DeSilva LB, DeSilva UL and Mahendran M. J Natio Sci Coun Srilanka., 7, 1979;1-10.

10. 10Ali M, Bhutani KK and Srivastava TN. Phytochemistry.,. 29(11), 1990; 3601.

11. Sirsi M. Indian J Pharmacol. 26(5), 1964;129.

12. Dhawan BN, Patnaik GK, Rastogi RP, Singh KK and Tandon JS. Ind J Exp Biol., 15,1977; 208.

13. Viqar U Ahmad, Muhammad Zubair, Muhammad A. Abbasi, Farzana Kousar, Farman Ullah, Naheed Fatima, and Muhammad. I. Choudhary. Phenolic Glycosides from Symplocos racemosa. Z. Naturforsch., 60b, 2005;1101 – 1104.

14. Rastogi R, Mehrotra B. Compendium of Indian medicinal plants. Vol. 2. New Delhi: C.S.I.R.; 1991; pp-1234.

15. Trop Med Parasitol. 1987 Jun; 38(2): 128-30 In vitro drug screening in isolated male Onchocerca gibsoni using motility suppression. Nowak M, Hutchinson GW, Copeman DB.

16. Parasitology. 1988 Apr;96 (Pt 2):323-36

17. Tropenmed Parasitol. 1981 Mar; 32(1): 31-4. Wiad Parazytol. 1986; 32(3): 303-11

18. Das AK, Bhattacharya S, Chatterjee GK, Chaudhuri SK. J Pharmacol Methods. 1988 Dec;20(4):323-7.

19. Z Parasitenkd. 1980; 63(3): 261-9 Jenkins DC, Armitage R, Carrington TS.

20. Parasitology. 1986 Apr;92 (Pt 2):425-30 Rapson EB, Chilwan AS, Jenkins DC

21. (Howes HL Jr. J Parasitol. 1971 Jun; 57(3): 487-93.)

22. Lichtensteiger CA et al; 1999 Gomez-Barrio A, Bolas-Fernandez F, Martinez-Fernandez

23. Sethi PD. HPTLC: Quantitative analysis of pharmaceutical formulations. 1st ed. New

24. Delhi: CBS Publisher; 1996. p. 44-57. Peter EW. Thin layer chromatography: A modern practical approach. UK: The royal

25. society of chemistry; 2005. p. 6-154.

26. Quinn DI, Day RO. Drug interactions of clinical importance: an updated guide. Drug Safety 1995; 12: 393–452.

27. Masimirembwa CM, et al. The effect of chloroquine on the pharmacokinetics and metabolism of praziquantel in rats and in humans. Biopharm Drug Dispos 1994; 15: 33–43

28. Metwally A, et al. Effect of cimetidine, bicarbonate and glucose on the

bioavailability of different formulations of praziquantel. Arzneimittelforschung 1995; 45: 516–18.

29. Jung H, et al. Pharmacokinetic study of praziquantel administered alone and in combination with cimetidine in a single-day therapeutic regimen. Antimicrob Agents Chemother 1997; 41: 1256–9.

30. Arnold, M.D., Harry L. (1968). *Poisonous Plants of Hawaii*. Tokyo, Japan: Charles E. Tuttle Co.. pp. 51. ISBN 0804804745.

31. Nirmal SA, Malwadkar G, Laware RB. Anthelmintic activity *glabra*. Songklanakarin J Sci Technol 2007; 29: 755-757.

Address for the Correspondence:
NARASIMHA RAO R[1]*
Professor,
[5]HITS COLLEGE OF PHARMACY,
Bogaram (V),Keesara(m)RR.(Dist)-501301
E.mail: rnrao007@yahoo.com