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PHYTOCHEMICAL SCREENING, TLC AND ANTI-HELMENTIC ACTIVITY OF *TRACHYSPERMUM ROXBURGHIANUNM* AND *ANTHEUM GRAVEOLENS* OF FAMILY APIACEAE

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ABSTRACT

The aim of present research is at phytochemical and the in-vitro comparative study of the anthelmintic activity of aqueous and ethanolic extracts of seeds of *Trachyspermum roxburghianunm* and *Antheum graveolens* using Indian adult earthworms (*Pheretima Posthuma*) at 2 different concentrations (10 and 20mg/ml) in two different vol. 10ml and 15ml respectively. The study involved the observation of the time of paralysis and the time of death of the worms. At the conc. of 20mg/ml in both vol. i.e. ten ml and fifteen ml the Ethanolic extracts showed more potent activities as compared to the standard drug Albendazole at two different concentrations (10 and 20mg/ml) volume taken 15ml. Ethanolic extract gives more potent result than aqueous extract when compare to standard. But aqueous extract has also shows anthelmintic activity. In conclusion, aqueous and ethanolic extracts of seeds of *Trachyspermum roxburghianun* and *Antheum graveolens* as an anthelmintic have been confirmed and further studies is suggested to discover the active principles responsible for the activity.

KEYWORDS

Trachyspermum roxburghianunm, Antheum graveolens, Phytochemical anthelmintic, *Pheretima posthuma* and Albendazole.

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INTRODUCTION

Ethnomedicine and phytomedicine are the studies of medicinal properties of plants[1]. Plants have the ability to synthesize a wide variety of chemical compounds that are used for important biological functions, and to defend them against attack from predators such as insects, fungi, and herbivorous animals. Many of these phytochemical have

beneficial effects on health when consumed by humans, and are also effective to treat human diseases. At least 12,500 such active compounds have been isolated so far, a number estimated to be less than 10% of the total. Phytochemicals mediate their effects on the human body via identical processes which are already well understood for the chemical compounds in conventional drugs. Herbal medicines are not different from conventional drugs in terms of their mechanism of action. This enables herbal medicines to be as effective as conventional medicines[2,3].

The use of plants as medicines predates written human history. Ethnomedicine is an effective way to discover future medicines. There are many compounds used in modern medicine which were derived from plant sources[4]. Many of the modern medicine currently available have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium. The World Health Organization estimates that 78-85% percent of the population of some Asian and African countries presently use treditioal herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available.

In India, Ayurveda medicine has been used from ancient time, many herbs such as turmeric possibly as early as 1900 BCE[5] Sanskrit writings from around 1500 BCE, such as the Rig Veda, are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system[6]. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BCE. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources[7].

The earliest known Greek herbals medicine record was "Diocles of Carystus", written during the 3rd century BCE, and one by Krateuas from the 1st century BCE Very few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals[8]. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates, "De herbs et curis" and in Galen's, "Therapeutics", provided the pattern for western medicine[9]. Sometime between 50 and 68 CE, a Greek physician known as Pedanius Dioscorides wrote "Peri hules iatrikes" commonly known by its Latin title "De Materia Medica" a 5 volume book describe more than 600 plants, 35 animal products, and ninety minerals and around 1000 medicine made by them. De Materia Medica remained the authoritative reference of herbalism into the 17th century[10]. Theophrastus's Historia Plantarum, written in between 350 BCE – 287 BCE in 10 volume of which 9 survive, it describes the plants by their uses and attempted a biological classification based on how plant reproduced, a first in the history of botany[11,12]. Many of the conventional drug available today have a long history as herbal remedies, including opium, aspirin, digitalis, and quinine.

The use of and search for new drugs and dietary supplements derived from plants have accelerated in recent years because the use of many conventional medicine produces toxicity also have potential side effects in human beings, many cases of resistance is also observed recently. Hence the development and discovery of new substances derived from plants which are considered to be the best source of bioactive substances. New researches are going on for the isolation of phytochemicals for the development of the new drug for the treatment of various diseases. .

Medicinal plants are the richest bio-resource of the traditional system of medicine, conventional medicine, and folk medicine, pharmaceutical intermediate and chemical entities for the new drug[13]. Plant are rich in secondary metabolites such as tannins, alkaloids, terpenoids, flavonoids etc. which possess anthelmintic and antimicrobial activities and may serve as an alternative, effective, cheaper and safe anthelmintic and antimicrobial

agents. A number of cases have been found with the use of a mixture of natural products use in the treatment of infections, most notably the synergistic effect and polypharmacological application of plant products[14]. Plants base anthelmintics have great therapeutic potential and have lesser side effect as compared to synthetic compounds and also has minimum chance of development of resistance. Therefore there is a need to search for new drugs from natural sources and to develop new infection-fighting strategies to control the situation.

Anthelmintic Activity

Antihelminthics are drugs that expel helminths (parasitic worms) from the body, by either stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing).

The word Helminths is derived from the Greek word "Helmins" which means "worm". Helminth are parasitic worms referring to various types of parasitic worms that live in the body. Soil-transmitted helminths infection is the most common infection, billion of people worldwide suffer from this infection[15]. *Ascaris lumbricoides, Trichuris trichiura, Nacator amiricanus* and *Ancylostoma duodenale* are the most common parasites[16].

Most people affected by these infection lives in less developed countries of Africa, South America, and South East Asia, where deprivation, along with poor sanitary conditions, give rise to infections with intestinal helminth. Although not mortal in most cases, these parasites can cause considerable morbidities, such as anemia and malnutrition, this leading to decreased growth and cognitive retardation, especially in children in endemic countries[17,18]. The World Health Organization disclosed that over 2.2 billion people are suffering from parasitic worm infections[19] and it is estimated that by the year 2025, about 58% of the population in developing countries will be influenced[20]. Effective treatments are desirable for all people affected by these parasites, the long-term potency remains undetermined and large-scale protective actions also bear the risk of resistances against the respective drugs to emerge[21-23]. This, in turn, will strongly limit the effective use of the very limited number of drugs against soil-transmitted helminths we are mainly relying on, namely albendazole, mebendazole, and levamisole[24]. At present the situation concerning resistances is not as severe as in veterinary medicine, monitoring of the drug potency should be improved and attempt in the development of new drugs be to speed up[25]. Natural products have always been a valuable source for the identification and the development of new drugs against various targets, including helminth[19,26]. One approach to discovering new drug is the investigation of plants based folk medicine, their traditional usage by an in-vitro verification of their respective bioactivity followed by advanced phytochemical studies leading to an isolation of the potential active principles[26].

Helminthes infections are now being conceded as the cause of many acute as well as chronic ill healths among the human beings as well as cattle[27]. In most developing and less developed countries, helminth infections are a major health concern because they make humans prone to other infections such as microbial infections[28]. Intestinal infections with helminthes can more easily treat than those infections that occur in other locations in the body because the worms need to be eliminated by the drug and the drug need not be absorbed when given by oral route[29]. Most of the anthelmintics used nowadays produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea[30]. Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections[31]. Because of the increasing anthelmintic resistance and the impact of regular or common anthelmintics on the environment, it is important to look for alternative approach against gastrointestinal nematodes[32]. Targets in helminths include processes - neuromuscular coordination, muscular activity, sensory processes, feeding and the regulation of coelomic pressure. There are some potential chemotherapeutic targets which include Energy metabolism, Nutrient uptake, Nucleic acid metabolism, anabolic pathways[33].

Mechanism of action of Anthelmintic drug

Drug resistance in human helminths

There is a lot of arguing on whether the reports on Drug Resistance in Human Helminths are real cases of resistance[35]. Many have made a detailed analysis of the data concerning *Necator americanus* and *Ancylostoma duodenale* and conclude that these reports are suggestive for the development of Drug Resistance, but fall short of providing conclusive evidence[36,37]. Concerning the suspected resistance to praziquantel in Senegalese strains of Schistosoma mansoni, in spite of suggestive laboratory and field trials, so far there is no real evidence of Drug resistance[38]. In-depth studies and models indicate that the low cure rates in Senegal may be explained by the specific epidemiological situation in the northern region of the country i.e. high initial worm burdens and high transmission intensities but also that currently available method would probably not detect a reduction of sensitivity of less than 5±10%. The resistance of *S. Mansoni* to oxamniquine in Brazil has been well documented[39,40] and there is some evidence that resistance to may be occurring in Egypt[41]. Development of resistance to ivermectin in Onchocerca volvulus has not yet been proved, but reduced susceptibility of microfilariae has been observed in some regions of the West-African Onchocercosis Control Programme[42]. Several authors have already warned of the development of resistance to ivermectin in large-scale programs against onchocerciasis which use the same drug continuously over many years[43,44] Recently, it has been highlighted to the possible development of drug resistance in adult macrofilariae, which would even be more disastrous than drug resistance in microfilariae[45]. Although the reports do not always provide conclusive evidence about emerging Drug resistance in human nematodes and schistosomes, the data indicate that tolerance traits are indeed present in some helminth.

Anthelmintic resistance is a serious problem in veterinary and appears to be developing in some helminths, importance to human health. Anthelmintic drugs are the only means of control of helminth infections in animals and humans and the continuous dependence on these pharmaceuticals will impose selection pressure for resistance development. There is a serious need for new drug development as anthelmintic. The problem of anthelmintic resistance is already very serious in nematode parasites. The recent reports on possible emerging drug resistance in human nematodes and schistosomes do not provide conclusive evidence for the increase of innately tolerant strains or for the appearance of newly mutated resistant strains. However, they strongly suggest that resistant strains can and do exist and that these strains may emerge more prominently under drug pressure (hookworm in Australia, schistosomes in Egypt) or under specific circumstances (schistosomes in Senegal)[46].

Human helminths with suspected or proven drug resistance

Necator americanus	→	Mebendazole	→ Mali
Ancylostoma duodenale	→	Pyrantel	→ Australia
Schistosoma mansoni	→	Oxamniquine	→ Brazil
Onchocerca volvulus	→	Ivermectin	→ West Africa

Plant Profile

Family Introduction – Apiaceae

The Apiaceae is also known as Umbelliferae and carrot family, are a family of mostly aromatic plants with hollow stems. The family is large, with more than three thousand seven hundred species spread across 435 genera. It is the 16th-largest family of flowering plants. Most of Apiaceae are annual, biennial herbs, with the leaves aggregated toward the base, though a minority are shrubs or trees. Their leaves are of variable size and alternately arranged, or alternate with the upper leaves becoming nearly opposite. The expound characteristic of this family is the inflorescence, a simple or compound umbel. Flowers across this family are fairly uniform and are usually perfect hermaphroditic and actinomorphic, but some are andromonoecious, polygamomonoecious, or even dioecious (as in Acronema). The flowers are nearly perfectly pentamerous, with 5 petals, sepals, and stamens.

Most members of the Apiaceae are promiscuous. This means that they can be pollinated by almost any critter that can walk over the surface of the

inflorescence. They are generally self-compatible, so most pollination is geitonogamous.

Trachyspermum roxburghianum

Trachyspermum roxburghianum is a plant in the family Apiaceae. Ajmoda is an erect, branched annual herb, 0.5-3 ft tall. Stems are longitudinally triped. Leaves are double-compound, ultimate segments all linear. Flowers occur in compound umbels. They have rounded white or pink petals. Fruits are ovoid, ultimately shining, yellow, 2-2.5mm long and hispid. It is a very strong spice, with a characteristic smell similar to parsley. Its aromatic dried fruits, like its close relative ajwain, are often used in Bengali cuisine. The fresh leaves are used as a herb and seeds are medicinally used. Flowering: December-February.

Common name

English : Wild Celery
Hindi : Ajmod
Tamil : Asamtavomam
Malayalam : Ayamodakam
Telugu : Ajumoda, Vamu
Kannada : Ajamodhavoma
Bengali : Randhuni, Shah jira
Urdu : Ajmod
Sanskrit : Ajamoda

Geographical Distribution

Trachyspermum roxburghianum native plant of tropical Asia and is cultivated in Bangladesh, India and Indo-China. The plant is cultivated throughout India, Cultivated in Madhya Pradesh, Andhra Pradesh, Gujarat, Maharashtra, Uttar Pradesh, Rajasthan, Karnataka and Tamil Nadu.

Part Use

Seeds of *Trachyspermum roxburghianum.*

Chemical constituents

Major component of seeds is limonene. Other constituents were sabinene, terpinen-4-ol, ligustilide and γ-terpinene are d-limonene, alpha-terpinene, d-linalool, dl-terpineol and dl-piperitone. Thymol content is 1.7%. The main component of seed oil was limonene. Other notable constituents were sabinene, terpinen-4-ol, ligustilide and γ-terpinene[47,48]. Fruits yield essential oil with d-limonene, α-terpinene, dipentene, d-linalool, terpineol, dl-piperitone, thymoquinol, thymol and a crystalline ketonic acid[49]. Fruits yield essential oil with d-limonene, α-terpinene, dipentene, d-linalool, terpineol, dl-piperitone, thymoquinol, thymol and a crystalline ketonic acid[48].

Uses

T. roxburghianum plant oils and extracts have been used for a wide variety of purposes for many thousands of years[49]. Leaves are used as an anthelmintic, antiseptic, Carminative and antibacterial activity. The fruit induced hyperactivity of the central nervous system in mice. It also exhibited activity against Entamoeba histolytica. They also exhibit powerful antispasmodic activity. The fruit which are left after the extraction of the essential oil showed pronounced cardiotonic activity. The oil produced marked diuretic effect in rabbits. It lowered blood pressure in dogs and rats. Fruits are used as a condiment, stimulant, and carmine[50]. The seeds are useful in hiccup, vomiting and pain in the bladder[51]. They form ingredients of carminative and stimulant preparations and are very useful in dyspepsia and flatulence. Fruits of *T. roxburghianum,* often used in Indian cuisine, are a kind of very strong spice with characteristic smell similar to parsley[49].

Anethum graveolens

Dill grows to 40-65cm, with slender stems and alternate, finely divided, softly delicate leaves. The ultimate leaf divisions are 1-2.5mm broad, slightly broader than the similar leaves of fennel, which are threadlike, less than 1 mm broad, but harder in texture. The flowers are white to yellow, in small umbels 2-8cm diameter. The seeds are 4-6mm long and 1mm thick, and straight to slightly curve with a longitudinally ridged surface[52,53].

Common name

English : Dill, Indian dill,
Hindi : sowa,
Sanskrit : shataahvaa, shatapushpa,
Tamil : sadakuppaj

Geographical Distribution

Cultivated all over India, Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal.

Part Use

Seeds of *Anethum graveolens*.

Chemical Constitutes

Dill oil contain volatile oil and about half of which is carvone other compounds are flavonoids, coumarins, xanthones, and triterpenes. Carvone is the major constituent. The oil from seeds, used for flatulence in children and enters into the preparations of gripe water. The oil is also antimicrobial and antifungal[54].

Uses

Fresh and dried leaf is used for prevention and treatment of the gastrointestinal tract kidney and urinary tract for spasms and sleep disorder. Fruit are mainly used as an aromatic, antipyretic, carminative and anthelmintic[55]. The oil from seed is well known remedy for flatulence in children and enter in to the preparation of gripe water[54].

PLANT MATERIAL COLLECTION, AUTHENTICATION AND EXTRACTION

Plant collection and Authentication

Trachyspermum roxburghianum and *Anethum graveolens* are collected from local market of Chandigarh, India and authenticated by Dr. Anita Mahiswar, HOD, Dept. of Botany, Govt. Digvijay Autonomous PG College Rajnandgaon C.G.

Seeds are collected and air dried then reduced to coarse powder.

Extraction

Extraction refers to processes for the isolation of the active ingredients from drug material. Maceration - In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc is pressed, and the combined liquids are clarified by filtration or decantation after standing.

Preparation of Extract

Aqueous extract

50gm coarse powdered drug was kept for maceration with 400ml of water for 72 hours in a closed flask. The extract filter by vacuum filtration. The filtrate was evaporated; an extract was dried and used.

Ethanolic extract

50gm coarse powder drug was kept for maceration with 400ml of Ethanol for 72 hours.The extract filter by vacuum filtration. The filtrate was evaporated in a rotary evaporator under reduced pressure until semi-solid extract was obtained; an extract was dried and used.

PHYTOCHEMICAL SCREENING, THIN LAYER CHROMATOGRAPHY AND RESULT

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods[56-59] and the result is shown in Table No.11 and Table No.12.

Alkaloid

Dragendorff's reagent

Alkaloid gives the reddish brown precipitate with Dragendroff's reagent.

Mayer's reagent

Alkaloid gives the cream color precipitate with Mayer's reagent.

Wagner's reagent

Alkaloids give the reddish brown precipitate with Wagner's reagent.

Hager's reagent

Alkaloid gives the yellow precipitate with Hager's reagent.

Tannic acid test

Alkaloids gives buff color with tannic acid.

Amino acid

Millon's test

To the test solution add about 2ml of Millon's reagent white precipitate indicate the presence of amino acid.

Ninhydrine Test

To the test solution add Ninhydrine solution, boil violet color indicates the presence of amino acid.

Proteins

Warming Test

Heat the solution over boiling water bath, protein gets coagulated.

Biuret test

To the test solution about 2ml add 2ml biuret solution violet color indicate the presence of protein.

Hydrolysis test

Hydrolyze the test solution with the hydrochloric acid or sulphuric acid then carry out Ninhydrine test for amino acid.

Xanthoproteic test

To the 5ml of the test solution, add 1ml of conc. Nitric acid and boil yellow precipitate formed. After cooling it add 40% Sodium hydroxide solution orange color formed.

Starch

To the Aqueous extract add week aqueous iodine solution blue color indicate the presence of starch, which disappears on heating and reappears on cooling.

Steroids and Triterpenoids

Libermann-Burchard test

Treat the test extract with few drops of acetic anhydeide boil and cool, then add conc. Sulphuric acid by the side of test tube brown color ring formed at the junction of two layers and the upper layer turn green which show the presence of Steroids, the formation of deep red color show presence of Triterpenoids.

Salkowski test

Treat the solution with few drops of conc. Sulphuric acid red colour at the lower layer shows the presence of steroids and yellow colour lower layer indicate the presence of Triterpenoids.

Sulphur powder test

Add a small amount of sulphur powder to the test solution it sinks at the bottom.

Carbohydrates- Aqueous extract

Molisch's test

To the test solution add few drop of alcoholic a-naphthol then add few drop of conc. Sulphuric acid through the side of test tube purple to the violet colour ring formed at the junction.

Barfoed's reagent

1ml of test solution heated with 1ml of Barfoed's reagent on a water bath if the red cupric oxide is formed, monosaccharide is present, Disaccharide on prolong heating may cause the reduction, owing to partial hydrolysis to monosaccharide.

Test for pentose

To the test solution adds an equal volume of hydrochloric acid containing a small amount of Phloroglucinol and heat red colour is produced.

Fehlings test

Filtrate were hydrolyse by using dil. HCl then neutralized with 5% solution of Sodium hydroxide and heat with Fehling's A & B solution formation of red colour precipitate indicates the presence of reducing sugar.

Glycoside

General test

Test A

Extract 200mg of the drug with 5ml of dil. Sulphuric acid by warming on water bath filter it then neutralized with 5% solution of Sodium hydroxide and add 0.1ml of Fehling's A and B solution till it becomes alkaline [test with pH paper] and heat on boiling water bath for 2 min note the quantity of red colour precipitate form and compare with of form in test B.

Test B

Aqueous extract 200mg of the drug instead of dil. Sulphuric acid boil, and after boil add equal amount of water instead of 5% solution of Sodium hydroxide used in test A Now add 0.1ml of Fehling's A and B solution till it become alkaline [test with pH paper] and heat on boiling water bath for 2 min note the quantity of red colour precipitate form and compare with precipitate form in test A. If precipitate of test A is greater test B then glycoside may be present. Since Test B represent the amount of reducing sugar in crude drug whereas test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

Anthraquinone glycoside

Modified Bortrager's test- Boil 200mg of test material with 2ml of dil. Sulphuric acid treat it with 2ml of 5% aqueous Ferric Chloride solution freshly prepared, for 5 min, shake it with the equal volume of chloroform, separate the lower layer of chloroform and shake it with half of its volume of

dil. Ammonia. A rose pink to the red colour produced in Ammoniacal layer.

Test for Hydroxy- Anthraquinone

Treat the test solution with potassium hydroxide solution red colour is produced.

Cardiac Glycoside

Keller-Killiani

Test-extract the drug with chloroform and evaporate to dryness. Add 0.4ml of glacial acetic acid containing a trace amount of Ferric Chloride. Transfers to a small test tube add carefully 0.5ml of conc. Sulphuric acid by the side of test tube Acetic acid layer turns blue colour.

Legal's test

Treat the solution with pyridine and add alkaline sodium nitroprusside solution red colour appear.

Baljet's test

Treat the solution with picric acid or sodium picrate orange colour is formed.

Coumarin glycoside

Place small amount of test sample in a test tube with a filter paper moisten with dil. Sodium hydroxide solution, place test tube over the water bath for several mins. Remove paper and expose to UV light the paper shows green fluorescence.

Cyanogenetic glycoside

Place 200mg of the drug in a conical flask and moisten with few drop of water. [There should be no free liquid at the bottom of flask as the test will not work because the hydrogen cyanide produce will dissolve in water rather than come off as a gas to react with the paper] moisten a piece of picric acid paper with 5% aqueous Sodium carbonate solution and suspend by mean of cork in the neck of flask. Warm gently at 39^{o}C, observe the change in colour Hydro-cyanide is liberated from Cyanogenetic glycoside by the enzyme activity and react with sodium picrate to form the reddish purple sodium isopurpurate.

Saponins

Froth test

Extract were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 min formation of 1cm layer of foam indicates the presence of saponin.

Foam test

0.5mg of extract was shaken with 2-3ml of water. If foam produces persist for 10 min. indicate the presence of saponin.

Flavonoid

Alkaline Reagent test

To the test solution add few drop of sodium hydroxide solution intense yellow colour formed which turn colorless an addition of few drop of dil. Acid [hydrochloric or sulphuric acid] indicate the presence of flavonoids.

Zinc hydrochloride test

To the test solution add a mixture of zinc dust & conc. HCl gives red colour in few min.

Diterpenes

The extract was dissolved in water add with 3-5 drop of copper acetate solution formation of emerald colour indicate the presence of diterpenes.

Volatile oil

To the thin section of drug add 2-3 drop of Sudan III sol. red colour obtain by globules indicate the presence of volatile oil.

Tannins and Phenolic compound

Gelatine test

To the test solution add 1%gelatine solution containing 10% sodium chloride precipitate formed.

Ferric Chloride

Treat the extract with few drop of Ferric Chloride solution blue colour appears if hydrostabal Tannins are present, Green colour appear if condense Tannins are present.

Test for catechin

Dip the match stick in the test solution then dry it and lastly moisten with conc. Hydrochloric acid. Then warm the stick near flame the colour of the wood changes - pink due to phloroglucinol [phloroglucinol is formed when catechin are treated with acid].

Test forchlorogenic acid

Treat the solution with aq. ammonia and expose to air, green colour is developed.

Naphthoquinones

Juglone test

To the chloroform extract add 2-3ml of ethyl ether with dil. Ammonia solution pink colour indicate Naphthoquinones.

Dam-Karrer test

To the chlorofomic plant extract + 10% potassium hydroxide solution blue colour develop.

Thin layer Chromatography TLC

TLC is a solid liquid form of chromatography were stationary phase is normaly a polar absorbent and mobile phase can be single solvent or combination of solvent. TLC is quick less expencive microscale technique that can be use to

Determine no. of components.

Verify the substance identification.

Monitor the progeress of a reaction determine appropriate condition for coloum chromatography

Analyze the fraction obtain by column chtomatography.

Thin Layer Chromatography was performed on precoated plates. The plates were prepared by using Silica Gel G for TLC, left overnight for air drying. These plates were activated by hot air oven at 100ºC for 1hr. The dried and activated plate is then developed in suitable solvents for rapid screening, chloroform: methanol in the ratio 4.5:0.5 is taken as solvent system. The plateswere put [run] in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by unaided eye and then by UV light at 254mm. Different colour bands were observed and corresponding Rf values are determined. R_f value ie Retardation faction value express in decimal and is calculated by the given formula[60-62].

$$Rf = \frac{distancetravelbyspot}{distancetravelbysolvent}$$

Chemicals Required

Silica Gel G for Thin Layer Chromotography, Chloroform, Methanol (All from Molychem).

Trachyspermum roxburghianum

Solvent system - chloroform: methanol

Ratio - 4.5: 0.5

R_f value = 0.921, 0.843, 0.718, 0.625, 0.562, 0.421, 0.343, 0.171, 0.093.

Anethum graveolens

Solvent system - chloroform: methanol

Ratio - 4.5: 0.5

R_f value = 0.843, 0.718, 0.671, 0.453, 0.421, 0.250, 0.187, 0.140

Result of Phytochemical Screening and Thin Layer Chromatography

Phytochemical Screening

The preliminary phytochemical screening of *Trachyspermum roxburghianum* and *Anethum graveolens* revealed the presence of alkaloids, amino acid, steroid, glycoside, cardiac glycoside, saponin, flavonoid, tannin and phenolic compound, presented in Table No.3. TLC also shows similar R_f value presented in Table No.4. This means same compounds are present in the seeds.

Further work will emphasize the isolation and characterization of compounds present in seeds of *Trachyspermum roxburghianum* and *Anethum graveolens*

Thin Layer Chromotography

The R_f value of all extract of plant seeds *Trachyspermum roxburghianum and Anethum graveolens* have been presented in Table No4.

R_f value

0.921 -*Trachyspermum roxburghianum,*

0.843- *Trachyspermum roxburghianum, Anethum graveolens.*

0.718- *Trachyspermum roxburghianum, Anethum graveolens*

0.625- *Trachyspermum roxburghianum.*

0.562 -*Trachyspermum roxburghianum*

0.421 -*Trachyspermum roxburghianum, Anethum graveolens.*

0.343- *Trachyspermum roxburghianum*

0.250 -*Anethum graveolens*

0.171- *Trachyspermum roxburghianum*

0.187- *Anethum graveolens.*

0.140- *Anethum graveolens,*

0.093- *Trachyspermum roxburghianum*

ANTHELMINTIC ACTIVITY

Preparation of Extract and Test sample

Aqueous extract

50gm coarse powder drug was kept for maceration with 400ml of water for 72 hr in a closed flask. The extract filter by vacuum filtration. The filtrate was evaporated and the extract was dried and used.

Ethanolic extract

50gm coarse powder drug was kept for maceration with 400ml of Ethanol for 72 hr. The extract filter by vacuum filtration. The filtrate was evaporated in a rotary evaporator under reduced pressure until semi-solid extract was obtained and the extract was dried and used.

The sample for the experiment was prepared by dissolving extract [Ethanolic and Aqueous extract] of each seed of plants *Trachyspermum roxburghianum and Anethum graveolens* in 2% Tween 80 normal saline suspension to make the concentration of 10 and 20mg/ml and the volume was adjusted to 10ml and 15ml.

Animal

Indian adult earthworms (*Pheretima posthuma*) were used to study the anthelminthic activity as these worms resemble both anatomically and physiologically to the intestinal round worms[63-65]. Indian earthworm [adult] - *Pheretima posthuma,* collected from moist soil and washed with normal saline to remove all faecal matter, the earthworms of 4-7cm in length and 0.1-0.2cm in width were used for experimental.

Reference standard and Chemicals

Standard Drug

Albendazole received from Ankur Drugs and Pharma Ltd. Unit 1 Manakpur, Solan [HP].

Chemicals

Tween®80 [Molychem, Mumbai], Normal Saline [nirlife] purchased from a local shop.

Standard Albendazole was dissolved in 2% Tween 80 normal saline suspension to make the concentration of 10 and 20 mg/ml and the volume was adjusted 15 ml. Normal saline was taken as control.

Experimental Method

Anthelmintic activity of Ethanolic extract and Aqueous extract from the fruit commonly known as seeds of plants *Trachyspermum roxburghianum* and *Anethum graveolens* were evaluated on Indian adult earthworms (*Pheretima posthuma*) . Indian adult earthworms (*Pheretima posthuma*) were used to study the anthelminthic activity as these worms resemble both anatomically and physiologically to the intestinal round worms[63-65], earthworms of 4-7cm in length and 0.1-0.2cm in width were used for experimental The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter, and are divided into the group of seven each containing five earthworms for each extract of all seeds.

All extract were dissolved in two percent Tween 80 normal saline suspension to make a concentration of 10 and 20mg/ml and the volume was adjusted to 10ml and 15ml. Standard Albendazole was dissolved in two percent Tween 80 normal saline suspension to make the concentration of 10 and 20mg/ml and the volume was adjusted 15ml. Normal saline was taken as control.

All the test sol. and standard drug sol. were prepared freshly before the commencement of the experiment. Washed earthworm are released in petri dish of different concentration [i.e. 10 and 20mg/ml] and different volume [i.e. 10 and 15ml].

The observation value is observed in five observations. Five worms of about same size per petri dish were used. The time taken for complete paralysis and death of individual worms were recorded. The time taken for worm to become complete motionless was noted as time of paralysis and time of death was concluded when the worms lost their motility when dipped in warm water [50°C] followed with fading away of their body color and by applying frequent outer stimuli which stimulate or induce movement in earthworm if alive. The mean of time of paralysis and time of death was recorded in Table No.14 and Table No.15 Aqueous and Ethanolic extract respectively.

The possible mechanism of action for phytochemicals

Result Anthelmintic Activity

Anthelmintic activity of aqueous and ethanolic extracts of seeds of o*Trachyspermum roxburghianum and Anethum graveolens* using Indian adult earthworms (*Pheretima posthuma*) at two different concentrations (10 and 20mg/ml) in two different volumes 10ml and 15ml respectively had been observed. The study involved the determination of the time of paralysis (P) and time of death (D) of the worms. At the concentration of 20mg/ml in both volumes i.e. 10ml and 15ml the Ethanolic extracts showed very significant activities as compared to the standard drug Albendazole two different concentrations (10 and 20mg/ml) volume taken 15ml. Aqueous extracts also showed anthelmintic.

Both aqueous and ethanolic extracts of seeds of plant *Trachyspermum roxburghianum and Anethum graveolens* as an anthelmintic have been confirmed and further studies are suggested to discover the active principles responsible for the activity.

The extracts of *Trachyspermum roxburghianum and Anethum graveolens* produced a significant anthelmintic activity in a dose-dependent manner are shown in Table 5 and Table No.6 of Aqueous and Ethanolic extract respectively, the potential of both the extract Aqueous and Ethanolic with respect to standard Albendazole. Photograph of the Experiment takan by Canon Powershot A1100 IS showing results of Anthelmintic Activity of Aqueous and Ethanolic extract are from Figure No.23 to Figure No.50.

Time is in Minutes

Photograph of the Experiment

Aqueous Extract

Experimental Images takan by Canon Powershot A1100 IS showing results of Anthelmintic Activity of Aqueous extract of seeds of plant *Trachyspermum roxburghianum and Anethum graveolens.*

Ethanolic Extract

Experimental Image takan by Canon Powershot A1100 IS showing results of Anthelmintic Activity of Ethanolic extract of seeds of plant *Trachyspermum roxburghianum and Anethum graveolens.*

DISCUSSION AND CONCLUSION

Discussion

The preliminary phytochemical screening of *Trachyspermum roxburghianum* and *Apium graveolens* revealed the presence of alkaloids, amino acid, steroid, glycoside, cardiac glycoside, saponin, flavonoid, tannin and phenolic compound.

The R_f value of all extract of plant seeds *Trachyspermum roxburghianum and Anethum graveolens* have been presented in Table No.4. Various phytochemical gives different R_f value as well as same R_f value is observed in respective plants seeds extract. From this it is concluded that some same phytochemical in these extract are present along with some different phytochemical. Further work will emphasize the isolation and identification of active compound.

TLC profiling of all extracts gives an impressive result that directing towards the presence of a number of phytochemicals. Various phytochemicals give different R_f values in the different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in the understanding of their polarity and also helps in selection of an appropriate solvent system for separation of pure compounds by column chromatography. A mixture of solvents with variable polarity in different ratio can be used for separation of the pure compound from plant extract. The selection of an appropriate solvent system for particular plant extracts can only be achieved by analyzing the R_f values of compounds in the different solvent system. In our study, the most suitable solvent system for analysis was shown to be chloroform: methanol with the largest discriminating power. This information will help in the further separation of the compound from these plant extracts.

The *in-vitro* assays to screen the anthelmintic properties of plant extracts has main advantages that they are less in cost and rapid result allow the

screening of plants at large scale . In addition, these tests measured the effect of anthelmintic activity directly on the parasites without interfering the internal physiological functions of the host[65]. The higher activity of the ethanolic extracts as compared to the aqueous extract can be credited to the presence of higher amounts of phenolic compound as compared to aqueous extracts. The more useful explanation for the reduction in activity of aqueous extract is due to the enzyme polyphenol oxidase, which degrades polyphenols in water extracts, whereas in ethanol they are inactive. Moreover, water is a better medium for the growth of the micro-organisms as compared to ethanol[80]. Higher concentrations of bioactive flavonoid compounds are seen in ethanol due to its higher polarity[81]. Also, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material[82].

Earthworms have an ability to move by ciliary movement. The outer most layer of the *Pheretima posthuma* is mucilaginous and made of complex polysaccharides due to which this layer is slippery allow the earthworm to move freely. Any damage to the mucopolysaccharide membrane will reveal the outer layer and this confined its movement and can cause paralysis and this action may lead to the death of the worm by causing damage to the mucopolysaccharide layer[83,84]. Anthelmintics generally kill worms by 2 action - either starving them to death / paralyzing them. As worms have no means of energy storing, they have to eat continuously to meet their metabolic needs. Any disruption in this process results in energy reduction and interfering with feeding for 24 hr. or less is sufficient to kill most adult parasites. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut[8].

Coming to the chemistry of nematode surface, it is a collagen-rich extracellular matrix (ECM) providing defensive cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-translational and post-translational modification prior to assembly into higher order complexes. The mammalian skin also consists largely of collagen in form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence makes the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal or microbial/enzymatic attacks. Similar type of reaction is expected to take place between the nematode cuticle (the earthworm) and the tannins of plants, possibly by linking through hydrogen bonding. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death[74]. The anthelmintic activities of crude extracts can also be explained in part by their partial lipophilic nature, which renders the cells leaky and thereby results in cell death[72].

The preliminary phytochemical screening of *Trachyspermum roxburghianum and Anethum graveolens* revealed the presence of alkaloids, amino acid, steroid, carbohydrate, glycoside, cardiac glycoside, saponin, flavonoid, tannin and phenolic compound. The anthelmintic activity of *Trachyspermum roxburghianum* and *Anethum graveolens* might be due to the presence of alkaloids, tannins, saponins and phenolic compound. The possible mechanism of action for phytochemicals are-

Tannins - The anthelmintic effects of tannins may be attributed to its capacity to bind free protein available in the tubes for larval nutrition and thus minimize nutrient availability that may resulted in larval starvation or decrease in gastrointestinal metabolism thus causing larval death. Increases supply of digestible proteins by animals by forming protein complex compound in rumen. Interferes with energy generation by uncoupling oxidative phosphorylation[85-87]. React with nematode's cuticle and toughens the skin thus leading to paralysis. Tannin may Causes a decrease in G.I. metabolism

resulting in secretion of mucous and chemicals harmful to parasite[71,15,72].

Alkaloids - Have ability to Intercalates with the DNA synthesis of parasites[66]. Acts on CNS and causes paralysis of worms[88]. Possess anti-oxidating effects, thus reduces nitrate generation which is useful for the synthesis of protein and hence interfering with local homeostasis of worms[68]. Transcuticular diffusion is a common means of transport to enter into helminth parasites[69] for non-nutrient and non-electrolyte substances in nematodes. It has also been shown that this route is predominant for the uptake of many broad spectrum anthelmintics by different nematode, cestode and trematode parasites. The possible explanation for better anthelmintic activity of ethanolic extract compared to aqueous extract on larvae and adults parasites could be due to easier transcuticular absorption[70]. Steroidal alkaloid oligoglycosides may inhibit the transfer of sucrose from the stomach to the small intestine which could diminish the support of glucose to helminthes along with its antioxidant effect which is capable of reducing the nitrate generation (which can be used in the protein synthesis) as well as the possible inflammatory effect induced by the extract in the gastric and intestinal mucosal which could interfere in local homeostasis which is necessary for the development of helminthes[67].

Phenolic Compound- Interface with the energy generation and Uncoupling the oxidative phosphorylation thus Interfere with glycoprotein of cell surface[73].

Saponins - The phytochemical screening of the extracts also shows the presence of saponins. Recent research addressed that the main biological activity attribute to saponins was their membrane permeabilizing property. The main actions considered were changes in membrane permeability and pore formation, which is similar to the common anthelmintic drugs such as praziquantel. That is, they would affect the permeability of the cell membrane of the parasites and causes vacuolation and disintegration of monogenea teguments[74,75].

Steroidal Alkaloid - Suppresses transfer of sucrose from stomach to small intestine, thus diminishing the support of glucose to the helminths[75].

The possible explanation for the more potent activity of the alcoholic extract compared to the aqueous extract on adult parasites in the current study could be due to easier transcuticular absorption of the alcoholic extracts into the body of the parasite than the aqueous extracts. In general, alcoholic extracts of plants contain some non-polar organic chemicals with lower polarity than the aqueous extracts, making them more lipid soluble than the aqueous extracts and hence better anthelmintic activity. Lipophilic anthelmintics have a greater capability to cross the external surface of the helminths than the hydrophilic compounds[89].

Albendazole are broad spectrum oral anthelmintic its mechanism of action is through inhibiting microtubule synthesis thus irreversibly imparing glucose uptake, as a result, intestinal parasites are immobilized or die slowly[90].

Table No.1: Scientific Classification of *Trachyspermum roxburghianum*

Kingdom	*Plantae*
Subkingdom	*Viridaeplantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Order	*Apiales*
Family	*Apiaceae (Umbelliferae)*
Subfamily	*Apioideae*
Genus:	*Trachyspermum*
Species	*Roxburghianum*
Botanical name	*Trachyspermum roxburghianum*

Table No.2: Scientific Classification of *Anethum graveolens*

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Super division	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Apiales*
Family	*Apiaceae (Umbelliferae)*
Genus	*Anethum L.*
Species	*Anethum graveolens L.*

Table No.3: Results of phytochemical screening of *Trachyspermum ammi, Trachyspermum roxburghianum* and *Anethum graveolens*

S.No	Test	*T. roxburghianum*		*A.greveolens*	
		Aq.	**E**	**Aq.**	**E**
Alkaloid					
1	Dragendorff's reagent	-	-	-	+
2	Mayer's reagent	-	+	+	+
3	Wagner's reagent	-	+	-	+
4	Hager's reagent	+	+	-	+
5	Tannic Acid Test	+	+	-	+
Amino Acid					
6	Millon's Test	+	+	+	+
7	Ninhydrine Test	+	+	+	+
Protein					
8	Warming Test	-	-	+	-
9	Biuret Test	-	-	+	+
10	Hydrolysis Test	-	-	+	+
11	Xanthoproteic Test	-	-	-	-
12	Starch	**-**	**-**	**-**	**-**
Steroid and Triterpenoids					
13	Libermann-Burchard Test	+	+	+	+
14	Salkowski Test	+ yellow	+ yellow	+ yellow	+ yellow
15	Sulfur Powder Test	+	+	+	+
Carbohydrate Aqueous Extract only					
16	Molish Test	+	-	+	-
17	Barford Test	+	-	+	-
18	Test For Pentose	+	-	+	-
19	Fehling's Test	+	-	+	-
Glycoside					
20	General Test	+	+	+	+
21	Modified Borntrager Test	+	+	+	+

22	Test for Hydroxy-Anthraquinone	-	-	-	-
Cardiac Glycoside					
23	Keller Killiani Test	+	+	+	+
24	Legals Test	+	+	+	+
25	Baljets Test	+	+	+	+
26	Coumarin Glycoside	**+**	**+**	**+**	**+**
27	Cynogenetic Glycoside	**-**	**-**	**-**	**-**
Saponin					
28	Forth Test	+	+	+	+
29	Fome	+	+	+	+
Flavonoid					
30	Alkaline Reagent Test	+	+	+	+
31	Zinc Hydro-cholride Test	+	+	+	+
32	Diterpens	-	-	-	-
33	Volatile Oil	+		+	
Tannins and Phenolic Compound					
34	Gelatine Test	+	+	+	+
35	Ferric Chloride Test	+ Green	+ Green	+ Green	+ Green
36	Test for catechin	-	-	-	-
37	Test for chlorogenic acid	-	-	+	+
Naphthoquinones					
38	Juglone Test	-	-	-	-
39	Dam-Karrer Test	-	-	-	-

+= Present, - = Absent

Table No.4: Common R_f value of the extract

S.No	*T.roxburghianum*	*Antheum graveolens*
1	0.921	
2	0.843	0.843
3	0.718	0.718
4		0.671
5	0.625	
6	0.562	
7		0.453
8	0.421	0.421
9	0.343	
10		0.250
11	0.171	
12		0.187
13		0.140
14	0.093	

Table No.5: Result of Anthelmintic activity of aqueous extract

S.No	Plant Name	Aqueous Extract				Aqueous Extract			
		10mg/ml volume taken 10ml		10mg/ml volume taken 15ml		20mg/ml volume taken 10ml		20mg/ml volume taken 15ml	
		Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death
1	T. roxburghianum	59.3424 ±0.232382	75.2182 ±0.399529	57.3048 ±0.211443	73.263 ±0.207204	36.39 ±0.218361	57.8728 ±0.283352	33.2372 ±0.192497	55.3298 ±0.211849
2	Anethum graveolens	55.6408 ±0.119178	78.3212 ±0.187736	53.1486 ±0.391145	76.2432 ±0.228239	44.302 ±0.119178	60.4446 ±0.151929	41.5494 ±0.290748	58.959 ±0.558656

Time is in Minutes

Table No.6: Result of Anthelmintic activity of Ethanolic extract

S.No	Plant Name	Ethanolic Extract				Ethanolic Extract			
		10mg/ml volume taken 10ml		10mg/ml volume taken 15ml		20mg/ml volume taken 10ml		20mg/ml volume taken 15ml	
		Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death
1	T. roxburghianum	7.9812 ±0.232104	22.4308 ±0.195423	6.5062 ±0.064134	21.442 ±0.093696	5.183 ±0.126161	19.9156 ±0.32482	4.45 ±0.158232	18.395 ±0.220993
2	Anethum graveolens	7.348 ±0.119178	24.2884 ±0.263141	6.3548 ±0.204841	22.3418 ±0.190727	5.4228 ±0.119178	20.2014 ±0.165254	4.3334 ±0.216336	17.4018 ±0.177327
3	Standard Albendazole	-	-	11.2872 ±0.221321	27.305 ±0.252536	-	-	7.1942 ±0.1187	22.4684 ±0.116991

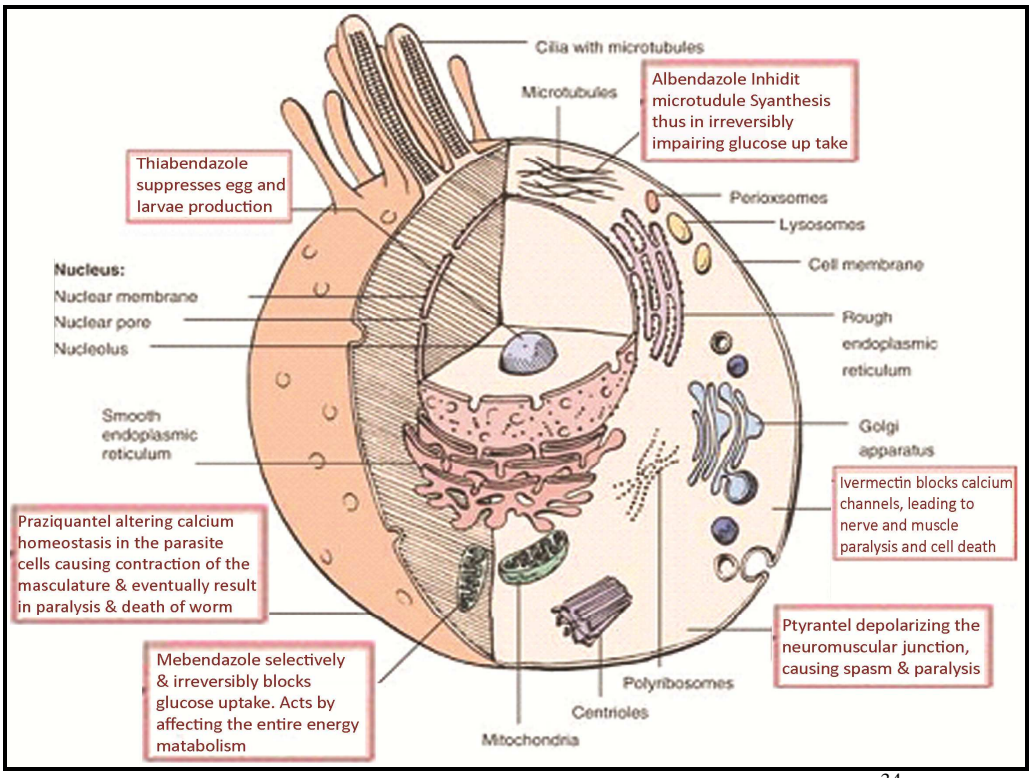

Figure No.1 Mechanism of action of Anthelmintic drug[34]

Figure No.2: *Trachespermum roxburgianum* [Ajmoda] seeds

Figure No.3: Ajmoda Leaves, fruit and Flower

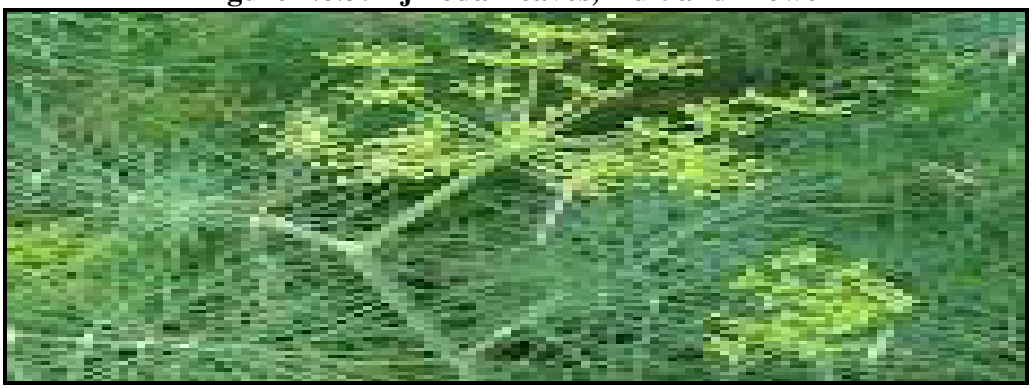

Figure No.4: *Anethum graveolens* plant

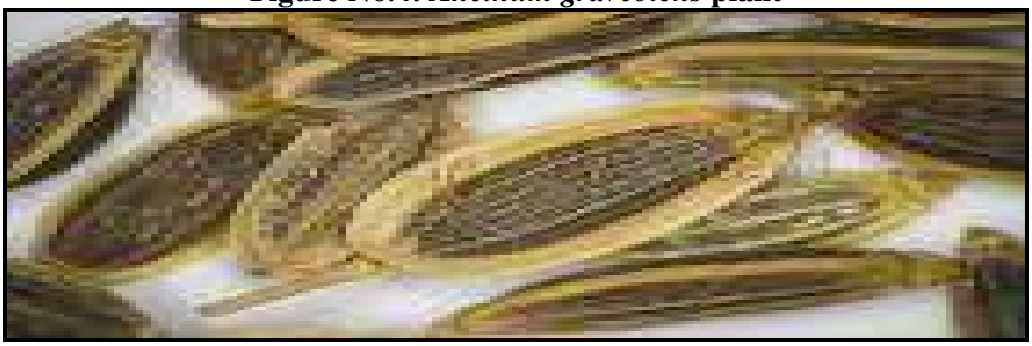

Figure No.5: *Anethum graveolens* seed

Figure No.6: TLC of *Trachyspermum roxburghianum*

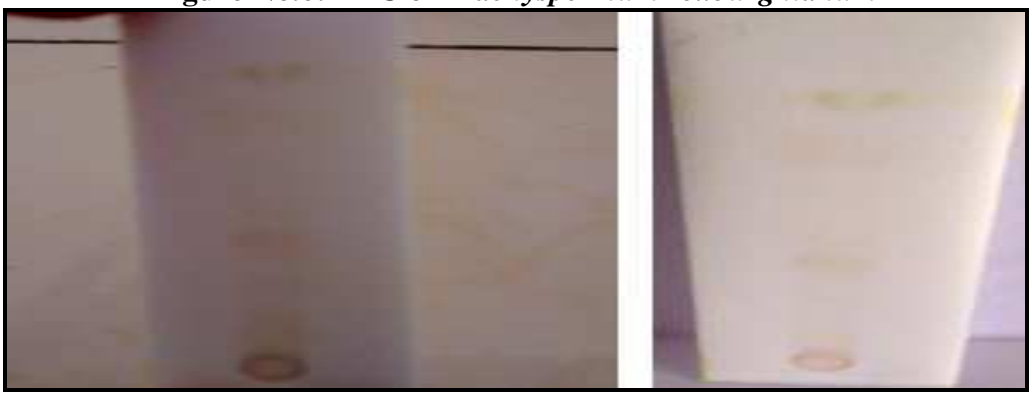

Figure No.7: TLC of *Anethum graveolens*

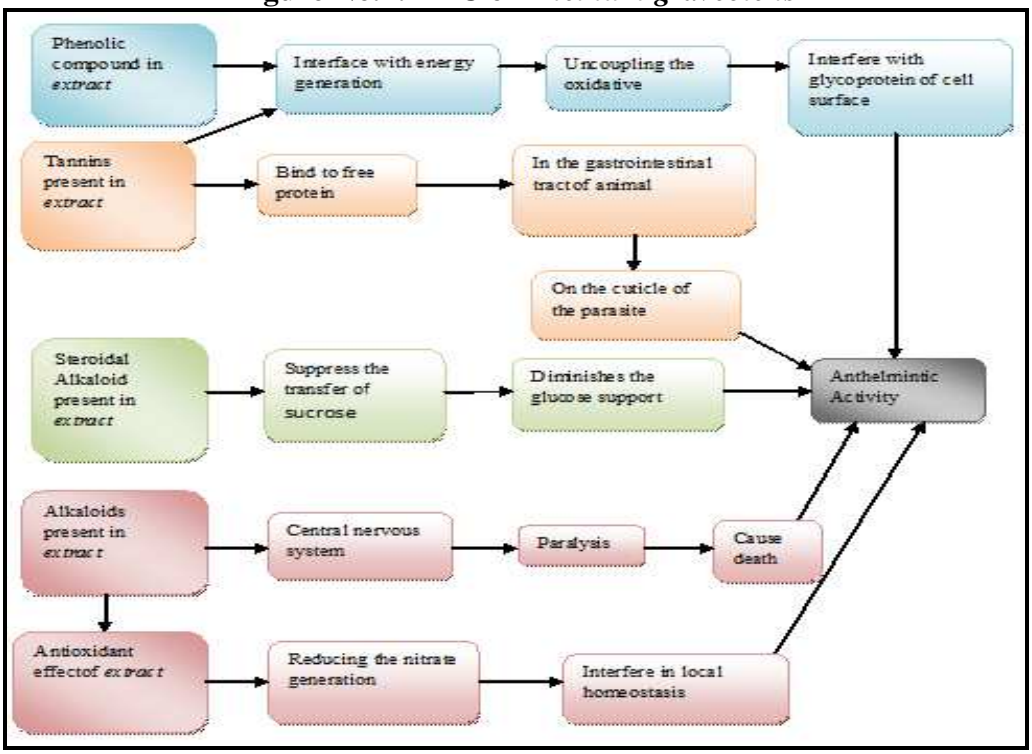

Figure No.8: Possible mechanism of Action of Phytochemical present in the extracts Anthelmintics[66-71,15,72-79]

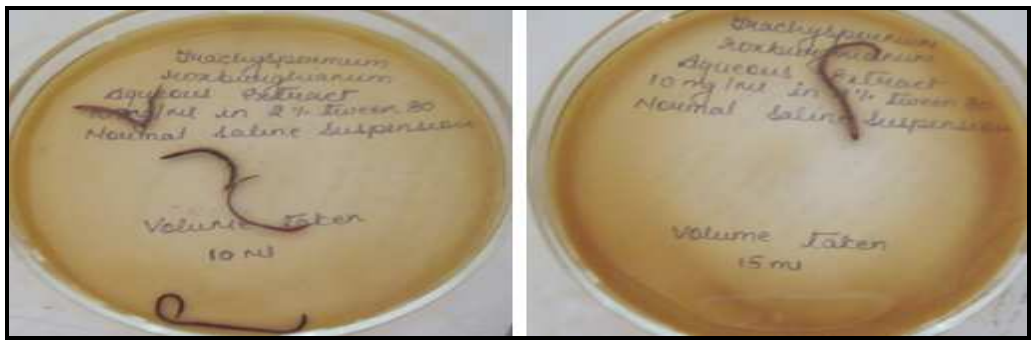

Figure No.9: Anthelmintic activity of aqueous extract of *Trachyspermum roxburghianum* 10mg/ml vol taken 10ml and 15ml

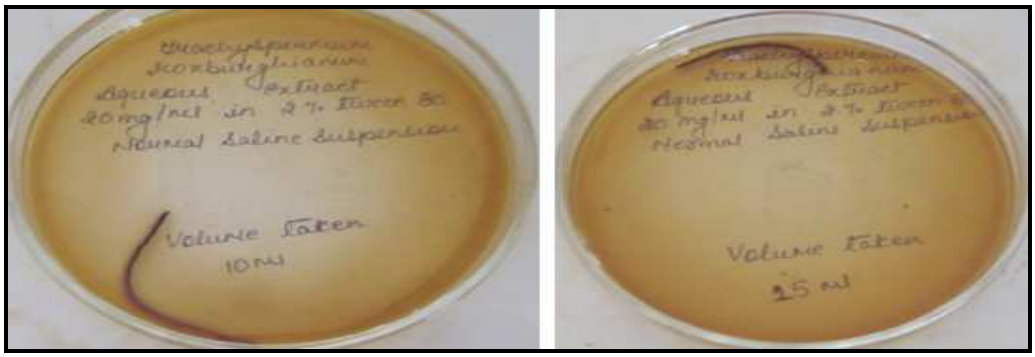

Figure No.10: Anthelmintic activity of aqueous extract of *Trachyspermum roxburghianum* 20mg/ml vol taken 10ml and 15ml

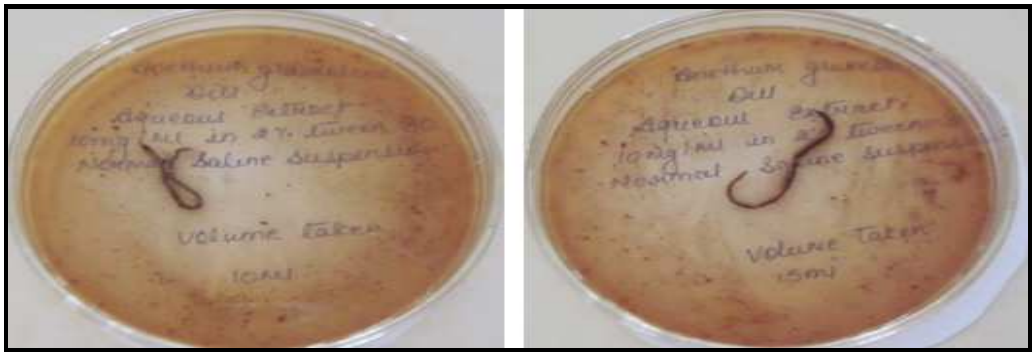

Figure No.11: Anthelmintic activity of aqueous extract of *Anethum graveolens* 10mg/ml vol taken 10ml and 15ml

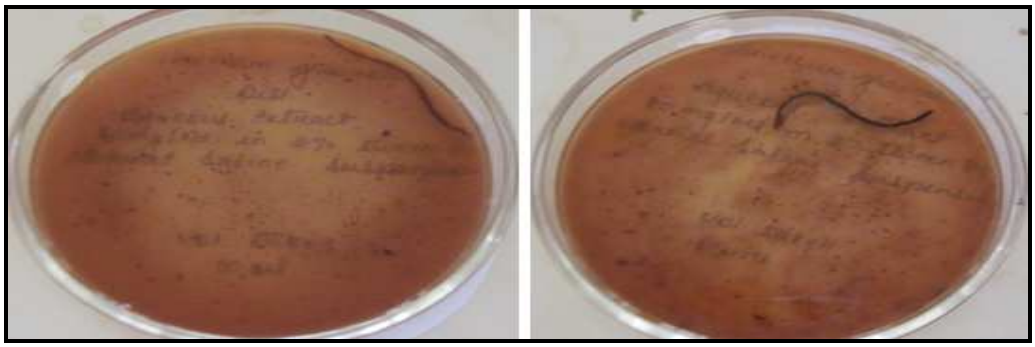

Figure No.12: Anthelmintic activity of aqueous extract of *Anethum graveolens* 20mg/ml vol taken 10ml and 15ml

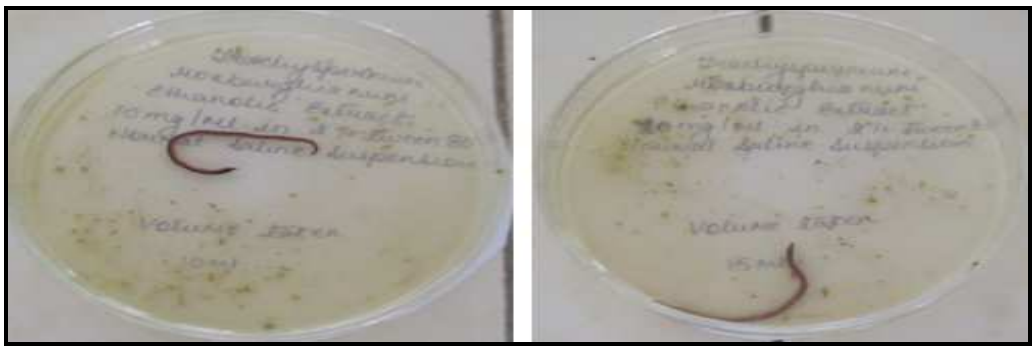

Figure No.13: Anthelmintic activity of ethanolic extract of *Trachyspermum roxburghianum* 10mg/ml vol taken 10ml and 15ml

Figure No.14: Anthelmintic activity of ethanolic extract of *Trachyspermum roxburghianum* 20mg/ml vol taken 10ml and 15ml

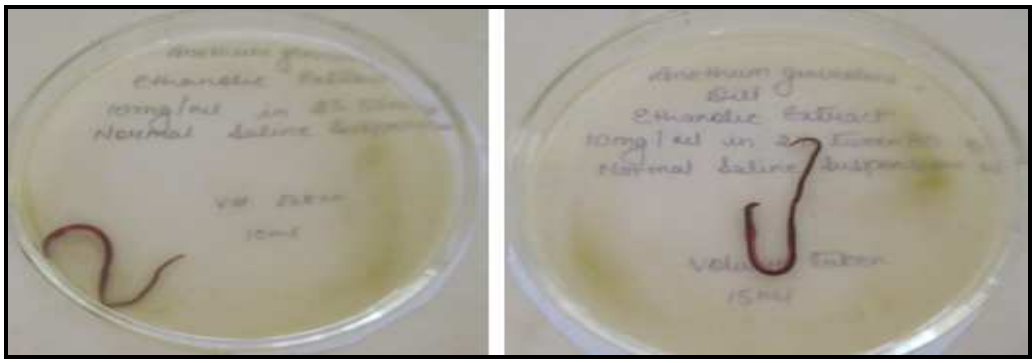

Figure No.15: Anthelmintic activity of ethanolic extract of *Anethum graveolens* 10mg/ml vol taken 10ml and 15ml

Figure No.16: Anthelmintic activity of ethanolic extract of *Anethum graveolens* 20mg/ml vol taken 10ml and 15ml

CONCLUSION

It can be concluded that active constituents responsible for anthelmintic i.e. alkaloids, amino acid, steroid, glycoside, cardiac glycoside, saponin, flavonoid, tannin and phenolic compound are present in the aqueous and ethanolic extracts of seeds of *Trachyspermum roxburghianum and Anethum graveole*.

Ethanolic extracts shows more potent activity than aqueous extract, the higher activity of the ethanolic extracts as compared to the aqueous extract can be credited to the presence of higher amounts of phenolic compound as compared to aqueous extracts. The more useful explanation for the reduction in activity of aqueous extract is due to the enzyme polyphenol oxidase, which degrades polyphenols in water extracts, whereas in ethanol they are inactive. Higher concentrations of bioactive flavonoid compounds are seen in ethanol due to its higher polarity. Also, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material.

Further work will emphasize the isolation and characterization of active principles responsible for anthelmintic and antimicrobial activity of seeds extracts of *Trachyspermum roxburghianum and Anethum graveolens*.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Lichterman B L. Aspirin: The story of a wonder drug, *British Medical Journal,* 329(7479), 2004, 1408.
2. Tapsell L C, Hemphill I, Cobiac L, *et al.* Health benefits of herbs and spices: The past, the present, the future, *Med. J. Aust,* 185(S4), 2006, 1-24.
3. Lai P K, Roy J. Antimicrobial and chemopreventive properties of herbs and spices, *Curr. Med. Chem,* 11(11), 2004, 1451-1460.
4. Fabricant D S, Farnsworth N R. The value of plants used in traditional medicine for drug discovery, *Environ. Health Perspect,* 109(1), 2001, 69-75.
5. Aggarwal B B, Sundaram C, Malani N, Ichikawa H. Curcumin: The Indian solid gold, *Advances in Experimental Medicine and Biology,* 595, 2007, 1-75.
6. Judith Sumner. The natural history of medicinal plants, *Tim Pre,* 17, 2000, 483-490.
7. Girish Dwivedi, Shridhar Dwivedi. History of Medicine: Sushruta - the clinician - Teacher par excellence, *National Informatics Centre,* 49(4), 2007, 243-244.
8. Robson, Barry, Baek. The engines of Hippocrates: From the dawn of medicine to medical and pharmaceutical informatics, *John Wiley and Sons,* 2009, 50.
9. Loudon, Irvine. Western medicine: An illustrated history, *Oxford University Press,* 2002, 54.
10. Minta Collins. Medieval herbals: The illustrative traditions, *University of Toronto Press,* 2000, 32.
11. Chisholm, Hugh. Theophrastus, encyclopædia britannica, *Cambridge University Press,* 11th Edition, 1911, 11.
12. Grene, Marjorie. The philosophy of biology: An episodic history, *Cambridge University Press,* 2004, 11.
13. Hammer K A, Carson C F, Rilay T V. Antimicrobial activity of essential oil and other plant extract, *Journal Applid Microbiol,* 86(6), 1999, 985-990.
14. Gibbons S. An over view of plants products as potential therapeutics, *Expert Opinion on Therapeutic Patents,* 13(4), 2003, 489-497.
15. Patel J, Kumar G S, Qureshi M S, Jena P K. Anthelmintic activity of Ethanolic extract of whole plant of *Eupatorium odoratum.* L, *Inter Jour of Phyto,* 2(2), 2010, 127-132.

16. World Health Organization, *Soil-Transmitted Helminth Infections,* Available online: http://www.who.int/mediacentre/factsheets/fs366/en/ (accessed on 26 January 2015).
17. Bethony J, Brooker S, Albonico M, Geiger S M, Loukas A, Diemert D, Hotez P J. Soil-transmitted helminth infections: Ascariasis, trichuriasis, and hookworm, *Lancet,* 367(9521), 2006, 1521-1532.
18. Lustigman S, Prichard R K, Gazzinelli A, Grant W N, Boatin B A, McCarthy J S, Basanez M G. A research agenda for helminth diseases of humans: The problem of helminthiases, *PLoS Negl. Trop. Dis,* 6(4), 2012, e1582.
19. Hotez P J, Brindley P J, Bethony J M, King C H, Pearce E J, Jacobson J. Helminth infections: The great neglected tropical diseases, *J. Clin. Investig,* 118(4), 2008, 1311-1321.
20. Ndjonka D, Rapado L N, Silber A M, Liebau E, Wrenger C. Natural products as a source for treating neglected parasitic diseases, *Int. J. Mol. Sci,* 14(2), 2013, 3395-3439.
21. Mulla W A, Thorat V S, Patil R V, Burade K B. Anthelmintic activity of leaves of *Alocasia indica* Linn, *International Journal of Pharm Tech Research,* 2(1), 2010, 26-30.
22. Humphries D, Nguyen S, Boakye D, Wilson M, Cappello M. The promise and pitfalls of mass drug administration to control intestinal helminth infections, *Curr. Opin. Infect. Dis,* 25(5), 2012, 584-589.
23. Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: Systematic review and meta-analysis, *J. Am. Med. Assoc,* 299(16), 2008, 1937-1948.
24. Vercruysse J, Albonico M, Behnke J M, Kotze A C, Prichard R K, McCarthy J S, Montresor A, Levecke B. Is anthelmintic resistance a concern for the control of human soil-transmitted helminths? *Int. J. Parasitol. Drugs Drug Resist,* 1(1), 2011, 14-27.
25. WHO. WHO Model list of essential medicines, *WHO: Geneva, Switzerland,* 18th Edition, 2013, 1-47.
26. Vercruysse J, Levecke B, Prichard R. Human soil-transmitted helminths: Implications of mass drug administration, *Curr. Opin. Infect. Dis,* 25(6), 2012, 703-708.
27. Barnabas B B, Mann A, Ogunrinola T S, Anyanwu P E. Screening for Anthelminthic activities from extracts of *Zanthoxylum zanthoxyloides, Neocarya macrophylla and Celosia laxa* against *ascaris* infection in rabbits, *International Journal of Applied Research in Natural Products,* 3(4), 2010, 1-4.
28. Mohamed A A El-Rahman, Ismail M Abdel-Nabi, Mohamed A O, Moustafa F M. Cytotoxic effects of albendazole, antiparasitic drug, on the liver of the rat: Subchronic study, *Egyptian journal of Biology,* 1(1), 1999, 16-29.
29. Dwivedi A, Dwivedi S, Sitoke A K, Patel R, Jhade D. Anthelmintic activity of a Polyherbal preparation, *Ethnobotanical Leaflets,* 13, 2009, 259-262.
30. Aremu O A, Fawole J C, Chukwujekwu M E, Light J F, Finnie J, Staden V. *In vitro* antimicrobial, anthelmintic and cyclo-oxygenase inhibitory activities and phytochemical analysis of *Leucosidea sericea*, *Journal of Ethnopharmacology,* 131(1), 2010, 22-27.
31. Velan S S, Suresh A, Suresh V, Kumar S N, Bright A J, Karthikeyan P. In vitro anthelmintic activity of *Samanea saman* (Merr) bark, *Journal of Pharmacy Research,* 3(12), 2010, 2977-2978.
32. Jabbar A, Raza M A, Iqbal Z, Khan M N. An inventory of the ethnobotanicals used as anthelmintics in the southern Punjab (Pakistan), *Journal of Ethnopharmacology,* 108(1), 2006, 152-154.
33. Iqbal Z, Lateef M, Ashraf M, Jabbar A. Anthelmintic activity of *Artemisia brevifolia*

in sheep, *Journal of Ethnopharmacology,* 93(2-3), 2004, 265-268.
34. Hennessy D R. Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds, *Veterinary Parasitology,* 72(3-4), 1997, 367-390.
35. Jain P, Singh S, Singh S K. Anthelmintic potential of herbal drug, *International Journal of Research and Development in Pharmacy and Life Sciences,* 2(3), 2013, 412-427.
36. Geerts S, Gryseels B. Drug resistance in human helminths: Current situation and lessons from livestock, *Clinical Microbiology Reviews,* 13(2), 2000, 207-222.
37. De Clercq D, Sacko M, Behnke J, Gilbert F, Dorny P, Vercruysse J. Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali, *American Journal of Tropical Medicine and Hygiene,* 57(1), 1997, 25-30.
38. Reynoldson J A, Behnke J M, Pallant L J. Failure of pyrantel in treatment of human hookworm infections (Ancylostoma duodenale) in the Kimberley region of NorthWest Australia, *Acta Tropica,* 68(3), 1997, 301-312.
39. Geerts S, Gryseels B. Anthelmintic resistance in human helminths: A review, *Tropical Medicine and International Health,* 6(11), 2001, 915-921.
40. Cioli D, Pica-Mattoccia L, Moroni R. Schistosoma mansoni: Hycanthoone/ oxamniquine resistance is controlled by a single autosomal recessive gene, *Experimental Parasitology,* 75(4), 1992, 425-432.
41. Cioli D, Pica-Mattoccia L, Archer S. Drug resistance in schistosomes, *Parasitology Today,* 9(5), 1993, 162-166.
42. Ismail A, Botros S, Metwally A. Resistance to praziquantel: direct evidence from Schistosoma mansoni isolated from Egyptian villagers, *American Journal of Tropical Medicine and Hygiene,* 60(6), 1999, 932-935.
43. Prechard R K, Bourguinat C, Geary T G, Shoop W L. Ivermectin resistance, *Parasitology Today,* 9(5), 1993, 154-159.
44. Geerts S, Coles G C, Gryseels B. Anthelmintic resistance in human helminths: Learning from the problems with worm control in livestock, *Parasitology Today,* 13(4), 1997, 149-151.
45. Grant W. What is the real target for ivermectin resistance selection in Onchocerca volvulus? *Parasitology Today,* 16(11), 2000, 458-459.
46. Prichard R K, Blackhall W J, Geary T G. Anthelmintic resistance in helminths of importance in animal and human health, *Parasitology,* 132(8), 2007, 1073-1076
47. Chopra R N, Nayar S L, Chopra I C. Glossary of Indian medicinal plants, *CSIR, India,* 1956, 245.
48. Kher C P. Medicinal plant; An illustrated dictionary, *Springer Varlag Heidelberg Publication,* 2004, 665-667.
49. Braj K M, Sikhibhushan D. Chemical examination of the essential oil derived from the seeds ofCarum Roxburghianum Benth, *Proc. Indian Acad. Sciences,* 16(2), 1942, 157-162.
50. Uphof J C, Verlag Von J. Kramer, Lehre. Dictionary of economic plants, *Germany,* 2, 1968, 591.
51. Kirtikar K R, Basu B D. Indian medicinal plants, *Lalit Mohan Basu Publications, Dehra Dun, Allahabad, India,* 2nd Edition, 1935, 624.
52. Grieve M. Dill, A Modern Herbal, *Botanical.com*, 2011.
53. Dill Weed. Our spices, *Olde Thompson Inc,* 2010.
54. Kher C P. Indian medicinal plant: An illustrated dictionary, *Springer Varlag Heidelberg Publication,* 2004, 124-125.
55. Pullaiah T. Medicinal plant in India, *Daya Books,* 1, 2002, 55.

56. Kokate C K, Gokhale S B. Text book pharmacognosy, *Nirali Prakashan,* 37, 593-594.
57. Roopashree T S, Dang R, Rani S R H, Narendra C. Antibacterial activity of anti-psoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis, *International Journal of Applied Research in Natural Products,* 1(3), 2008, 20-28.
58. Obasi N L, Egbuonu A C C, Ukoha P O, Ejikeme P M. Comparative phytochemical and antimicrobial screening of some solvent extracts of Samanea saman pods, *African Journal of Pure and Applied Chemistry,* 4(9), 2010, 206-212.
59. Audu S A, Mohammed I, Kaita H A. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae), *Life Science Journal,* 4(4), 2007, 75-79.
60. Harborne J B. Phytochemical methods of analysis, *Jackmann and Hall, London,* 1973, 64-190.
61. Kokate C K. Practical pharmacognosy, *Vallabh Prakashan, New Delhi,* 3rd Edition, 1994, 07.
62. Wagner H and Bladt S. Plant drug analysis - A thin layer chromatography atlas, *Thompson Press Ltd, New Delhi,* 2nd Edition, 2004, 384.
63. Thorn *et al*. Harrison's principles of internal medicine, *Mc Graw Hill Co, New York,* 1977, 1088-1089.
64. Viger Z. Atlas of medicinal parasitology, *P.G. Publications House, Singapore,* 3, 1984, 216-217.
65. Vidyarthi R D. A textbook of zoology, *S.Chand Co, New Delhi,* 1967, 132.
66. Feven B, Biniam G, Debesai G, Atul K. Screening of crude extracts of medicinal plants used in Eritrean unorthodox medicine for anthelmintic activity, *The Pharma Research,* 4, 2010, 133-137.
67. Roy H. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC, *Journal of Pharmaceutical Science and Technology,* 2(5), 2010, 217-221.
68. Borba H R, Freire R B, Albuquerque, A C, Cardoso M E O, Braga I G, Almeida S T P, Ferreira M J C, Fernandes G L T, Camacho AC L, Lima R C, Almeida A C C, Mattos D M M, Duarte R M, Nascimento S F, Framil R A, Dire G F. Anthelmintic comparative study of *Solanum lycocarpum* St. Hill extracts in mice naturally infected with *Aspiculuris tetraptera, Nature and Science,* 8(4), 2010, 94-100.
69. Shaibani T R M A, Phulan M S, Shiekh M. Anthelmintic activity of *Fumaria parviflora* (Fumariaceae) against gastrointestinal nematodes of sheep, *Int. J. Agric. Biol,* 11(4), 2009, 431-436.
70. Geary G T, Sangster N C, Thompson D P. Frontiers in anthelmintic pharmacology, *Veterinary Parasitology,* 84(3-4), 1999, 275-295.
71. Bachaya H A, Iqbal I, Khan M N, Jabbar J, Gilani A H Din I U. *In vitro* and I*n vivo* anthelmintic activity of *Terminalia Arjuna* bark, *Int. J. Agric. Biol,* 11(3), 2009, 273-278.
72. Vidyadhar S, Saidulu M, Gopal T K, Chamundeeswari D, Rao U, Banji D. *In vitro* activity of the whole plant of *Enicostemma littorale* by using various extracts, *International Journal of Applied Biology and Pharmaceutical Technology,* 1(3), 2010, 1119-1125.
73. John J, Mehta A, Shukla S, Mehta P. A report on anthelmintic activity of *Cassia tora* leaves, *J. Sci. Technol,* 31(3), 2009, 269-271.
74. Wang G S, Han J, Zhao L W, Jiang D X, Liu Y T, Liu X L. Anthelmintic activity of steroidal saponins from *Paris polyphylla*, *Phytomedicine,* 17(14), 2010, 1102-1105.
75. Cruz A S P. Anthelmintic effect of *Solanum lycocarpum* in mice infected with *Aspiculuris tetraptera*, *The Journal of American Science,* 4(3), 2008, 75-79.

76. Jain D, Maheshwari D, Somani R. *Journal of Advances in Drug Research,* 1(2), 2011, 965-967.
77. Chandrasekhar C H, Latha K P, Vagdevi H M, Vaidya V P. *International Journal of Green Pharmacy,* 2(2), 2008, 100-103.
78. Scalbert A. Antimicrobial properties of tannins, *Phytochem,* 30(12), 1991, 3875-3883.
79. Athanasiadou S, Kyriazakis I, Jackson F, Coop R L. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and *in vivo* studies, *Vet. Parasito,* 99(3), 2001, 205-219.
80. Shaibani I R M A, Phulan M S, Arijoand A, Qureshi T A. Ovicidal and larvicidal properties of *Adhatoda vasica* (L.) Extracts against gastrointestinal nematodes of sheep *in vitro*, *Pakistan vet. J,* 28(2), 2008, 79-83.
81. Lapornik B, Prosek M, Wondra A G. Comparison of extracts prepared from plant by products using different solvents and extraction time, *Journal of Food Engineering,* 71(2), 2005, 214-222.
82. Bimakr M. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves, *Food Bioprod Process,* 89(1), 2010, 1-6.
83. Fabricant D S, Farnsworth N R. The value of plants used in traditional medicine for drug discovery, *Environ. Health Perspect,* 109(1), 2001, 69-75.
84. Wang G X. *In vivo* anthelmintic activity of five alkaloids from *Macleaya microcarpa* (Maxim) Fedde against *Dactylogyrus intermedius* in *Carassius auratus*, *Veterinary Parasitology,* 171(3-4), 2010, 305-313.
85. Susan schoenian. Understanding anthelmintics, Small ruminant info sheet, 2010.
86. Sutar N, Garai R, Sharma U S, Sharma U K. Anthelmintic activity of *Platycladus orientalis* leaves extract, *International Journal of Parasitology Research,* 2(2), 2010, 1-3.
87. Mute V M. Anthelmintic effect of *Tamarind indica* linn leaves juice exract on *Pheretima posthuma*, *International Journal of Pharma Research and Development,* 7, 2009, 1-6.
88. Sharma U S, Sharma U K, Singh A, Sutar N, Singh P J. *In vitro* anthelmintic activity of *Murraya koenigii* linn. Leaves extracts, *International Journal of Pharma and Bio Sciences,* 1(3), 2010, 1-4.
89. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. *In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus, Journal of Ethnopharmacology,* 110(3), 2007, 428-433.
90. Jay S K, John M K. Mebendazole, *Annals of Internal Medicine,* 91(4), 1979, 582-586.

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