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PHYTOCHEMICAL INVESTIGATION, ANTHELMINTIC AND ANTI-MICROBIAL ACTIVITIES OF BETEL LEAF

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ABSTRACT

Many herbal extractions have been recommended for the cure of different diseases. The present research deals with phytochemical investigation, anthelmintic and anti-microbial activities of betel leaf collected from the local garden. Standardization of crude drug extraction from plant plays an important role in identifying the quality and purity of drugs. The leaves of the plant are extracted by Soxhlation using methanol as solvent. Then this extraction distilled to get a concentrated mass. Phytochemical investigation shows the presence of alkaloids, terpenoids, glycosides, proteins, and tannins in the extract of piper betel. In-vitro anthelmintic activity was observed by Indian earthworms, Pheretima posthuma having anatomical and physiological resemblance with intestinal roundworms. Before using these earthworms, washed in normal saline solution. To determine anthelmintic activity, all the investigations were carried out with different concentrations of 50, 100, 150, 200 µg/ml, significant activity were reported by time of paralysis and time of death. Antimicrobial activity was also determined, these compounds exhibit a maximum zone of inhibition against Bacillus subtilis. This study gives the way for further studies should be undertaking to elucidate the extract mechanism of action by which extract exert their antibacterial effects.

KEYWORDS: Piper betel, Methanolic extract, Anti-microbial activity and Anthelmintic activity.

INTRODUCTION

Piper betel Linn., (family Piperaceae) commonly known as the betel vine is an important medicinal and recreational plant in Southeast Asia. Piper betel leaves shows inhibitory activity against oral cavity pathogens, aqueous extract shows its antibacterial effect towards Streptococcus mutans, and other gram +Ve, gram -Ve organisms, skin antiseptic activity, anti- oxidative, anti-hemolytic activity and anti-malarial activity. The anti-bacterial activity of various solvent extracts of leaves was evaluated against the human pathogenic bacteria Escherichia coli, Klebsiella pneumonia, Bacillus subtilis Bacillus cereus, Salmonella typhi, Enterobacter aerogenes and Staphylococcus aureus by agar cup diffusion method. Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The world health organization estimates that a staggering 2 billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting of domestic pets indeed, the companies undertaking drug discovery programmes. Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Their advent changed the outlook of the physician about the power drugs can have on diseases. They are one of the few curative drugs. Their importance is magnified in the developing countries, where infective diseases predominate. As a class, they are one of the most frequently used as well as misused drugs. Drugs in this class differ from all others in that they are designed to inhibit/kill the infecting organism and to have no/minimal effect on the recipient. This of therapy is generally called chemotherapy which has come to mean `treatment of systemic infection with specific drugs that selectively suppress the infecting microorganism without significantly affecting the host. The basis of selective microbial toxicity is the action of the drug on a component of the microbe (e.g. bacterial cell wall) or metabolic processes (e.g. folate synthesis) that is not found in the host, or high affinity for certain microbial biomolecules (e.g. trimethoprim for bacterial dihydrofolate reductase).

MATERIAL AND METHODS

Collection of plant materials: The plant Piper betel was collected from the Garden area of Sathupally.

Extraction of drug:

The dried powdered plant material of Piper betel was extracted with methanol using soxhlation extraction method. After exhaustive extraction the collected extract was subjected to evaporation to obtain the pure drug of extract.

PHYTOCHEMICAL SCREENING

To perform the phytochemical screening the weighed amount (1gm) of powdered form of leaf of piper betel was taken. The leaves were soxhlated for 12 hours by using methanol as a solvent. After 12 hours they were filtered and the filtrates were obtained. These filtrates were further concentrated and isolated in ethyl acetates and ethanol and the drug were used to perform the phytochemical screening. The extracts were screened for the presence of various Phyto-constituents using the different chemicals and reagents.

Tests for Carbohydrates:

Molisch's Test: To 2-3 ml aqueous extract, add few drops of alpha- naphthol solution in alcohol shake and add conc.H2SO4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Iodine test: To 2-3 ml aqueous extract, add few drops of iodine solution and shake well. Black or blue color appears.

Benedict's test: Mix equal volume of Benedict's reagent and test solution. Heat for 2 -5 minutes in boiling water bath. Red or orange or yellow ppt is observed.

Test for Glycosides:

Legal's test: To aqueous extract, add 1ml pyridine and 1ml sodium nitroprusside. Pink to red color appears.

Test for Phyto steroids:

Salkowski test: To 2 ml of extract, add 2ml of chloroform and 2ml of concentrated H_2SO_4 shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Test for Anthraquinones :

To the 5 ml of extract add 2 ml of concentrated nitric acid. Pink to red was appears.

Test for Flavonoid:

Shinoda test: To dry power or extract' add 5ml 95% ethanol, few drops of conc.HCL and 0.5mg magnesium turnings. Orange, pink, red to purple colour appears.

Sulphuric acid test: On addition of sulphuric acid (60%-80%) flavones dissolve into it and give a deep yellowish solution.

Test for Alkaloids:

Dragendroff's test: To 2-3ml filtrate, add few drops Dragendroff's reagent. Orange brown ppt is formed

Mayer's test: To 2-3ml of filtrate add few drops of Mayer's reagent. White creamy ppt appears.

Hager's test: To 2-3ml of filtrate add few drops of Hager's reagent. Yellow ppt is observed.

Wager's test: To 2-3ml of filtrate add few drops of wager's reagent. Reddish brown ppt is observed.

Test for Terpenoids:

Salkowski test: To 5 ml of extract,add 2 ml chloroform and 3 ml of concentrated H_2SO_4 to form a layer .Greenish red colour was appear.

Test for Tannins:

5% FeCl3: To 2-3ml of aqueous extract, add few drops of 5% FeCl3 reagent. Deep blue-black is observed.

Gelatine solution test: To 2-3ml of aqueous extract; add few drops of gelatine solution gives White ppt.

Preparation of broth culture:

For the preparation broth culture of used bacteria, the liquid media was prepared as per given composition for broth culture. After the sterilization of media, the bacterial strains were inoculated under laminar air flow. The incubation of inoculated media was carried out at 37ºC for 48hours.

Antibacterial activity

The antibacterial activities of the extracts were determined by agar cup plate method.Nutrient agar medium was used for the test. Under aseptic conditions in the laminar air flow chamber nutrient agar medium was dispensed into pre sterilized petri dishes to yield a uniform depth of 4mm. The media was allowed to solidify. The test microorganisms were seeded into media containing petri dishes by spread plate method (100µl) with 24 hours cultures of bacteria. The plates were kept for pre diffusion for 15 minutes before use. Wells were then punched with a sterile cork borer (6mm) diameter and 50µl of the extracts (10,20,40µg/ml in DMSO) were placed into each well. Finally, the plates were incubated for 18- 24 hours at 37◦c. The diameter of zone of inhibition [mean of triplicates +or – S.D] was indicated by clear area which was devoid of growth of microbes was measured.

Minimum inhibitory concentrations:

The minimum inhibitory concentrations (MIC) for the most active extracts were recorded after 24hours. The extracts were diluted to get concentration ranging from 10,20,40mg/ml. After sterilization the media was dispensed into petri plates and was inoculated with 24 hours culture of each organism with a sterile cork borer (6mm). Wells were prepared and different crude extracts ranging from 10-40mg/ml were added to the wells and controls were maintained without plant extract. Inhibitions of organism growth in the plate containing test crude extracts were judged by comparison with growth in blank.

ANTIHELMINTIC ACTIVITY

Methods of collection of earthworms (pheretima posthuma) The appropriate time for their collection was found early in the a.m. in the summer, and noontime during the winter. Freshly collected alive worms were stored in the plastic bags, filled with suitable quantity of wet compost soil.

Evaluation of anthelminthic activity: An Indian adult earth worm 4-5 cm in length and 0.1-0.2 cm in width were used for the in vitro anthelmintic bio assay. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro (Sollmann 1918). The anthelmintic assay was carried out as per the method. (Ajaiyeoba et al 2001). The assay was performed in vitro using adult earthworm (Pheretima posthuma) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity.

The earthworms were divided into different groups containing two earth worms in each group.

The leaves of ethanol extract of piper betel were dissolve in water to give 50, 100, 150 and 200 µg/ml respectively. Albendazole was used as the standard at 100 µg/ml. 10ml of freshly prepared each extract of piper betel, standard solution were poured into Petri dish. The worms were washed with saline and released into the Petri dish and the time taken for the worms to get paralyzed and killed was noted. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worm neither moved when shaken vigorously nor when dipped in warm water (50ºC).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of plants was predominant to the detection of bio active principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs in the present study the presence of, phytochemicals were screened with the methanol extract of the piper betel leaf and the results are shown in table 1. Crude extracts and medicines are manufactured based on the principles of natural compounds even by pharmaceutical companies, may lead to large scale exposure of human to natural products. This procedure is simple for preliminary prerequisite before going to phytochemical investigation. Hence, in the present work, the crude extracts obtained by methanol as a solvent was screened for the presence of phytochemicals. The methanol extract shows the presence of anthraquinones, proteins, steroids, alkaloids, terpenoids, glycosides and tannins. Alkaloids have health benefits such as lower cholesterol, antimicrobial, anti-inflammatory and anticancer properties.

Table 1: Preliminary phytochemical screening of piper betel leaves.

TEST	METHANOL
Alkaloids	+
Carbohydrates	_
Tannins	+
Anthraquinones	+
Phyto steroids	+
Flavonoids	_
Terpenoids	+
Cardiac glycosides	+
Proteins and amino acid	+

(+) Indicates the presence of the phytochemical

(-)Indicates the absence of the phytochemical

Fig. 1: Phytochemical Screening of Methanolic Extract of Piper Betel Leaves.

Alkaloids are secondary metabolite having various pharmacological properties such as anti-oxidative, antifungal, anti-inflammatory and diuretic actions. The mechanism for anthelmintic activity of plant extracts are due to presence of secondary metabolites bind to free proteins in the gastrointestinal tract of host animal and glycoprotein on the cuticle of the parasite. The result of anthelmintic activity on earthworm pheretima posthuma was shown inTable-2 reveals that, different concentrations used have shown paralysis and death of worms and it was compared in the same concentration with piperazine citrate. This standard drug may cause hyper polarization of worm's muscle by GABA agonistic action opening chloride ion channels that cause relaxation and depresses responsiveness to contractile action of acetylcholine. By increasing chloride ion conductance of worm muscle membrane initiates hyper polarization and reduced excitability that led to muscle relaxation and flaccid paralysis.

Table: 2 Anthelmintic activity of Methanolic extract of PIPER BETAL LEAVES.

Groups	**Dose in concentration (µg\ml)**	**Time of paralysis (min)**	**Time of death (min)**
Control	-	-	-
Methanolic extract	50 µg\ml	2.05+/-0.62	3.10+/-0.85
	100 µg\ml	1.15+/-0.53	2.35+/-0.66
	150 µg\ml	1.00+/-0.34	2.05+/-0.55
	200 µg\ml	0.53+/-0.41	1.40+/-0.63
Standard drug	100 µg\ml	1.02+/-0.81	2.28+/-0.52

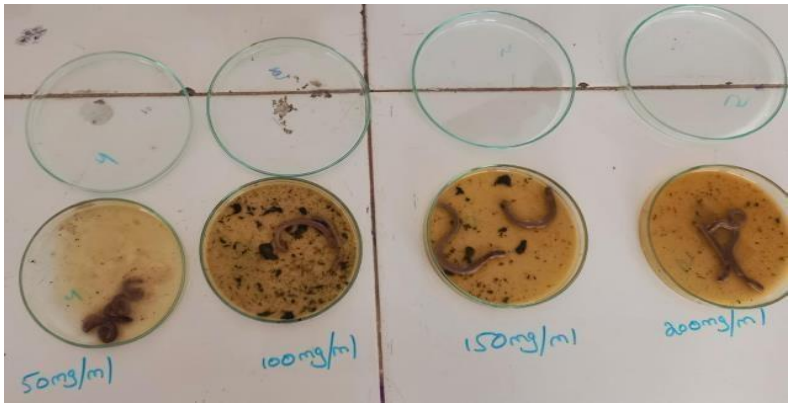

Fig 3: Anthelmintic activity of Methanolic extract of piper betel leaves

Fig 4: Anthelmintic activity of standard drug.

Table 3: Antimicrobial activity of Methanolic extract of Piper betel leaves.

S. NO	Name of organism	Conc.µg/ml	Zone of inhibition	Cefixime
			ZOD (mm)	ZOD (mm)
1	Bacillus subtilis	10 µg\ml	4.5mm	4.9mm
		20 µg\ml	4.8mm	5.1mm
		40 µg\ml	5.1mm	5.4mm

The results of anti-bacterial screening by agar cup plate method indicate the highest antibacterial activity was shown by the aqueous extract of leaf against the Bacillus subtilis. Standard antibiotic Cefixime was effective against all organisms and showed a zone of inhibition of 22-25mm. The results of the investigation showed that the leaf extract from piper betels have good antibacterial activity (10,20, and 40µg/ml) against Bacillus subtilis due to presence of alkaloids and Terpenoids.

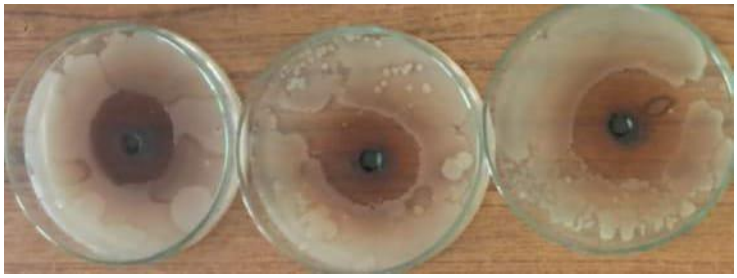

Fig. 5: Antimicrobial activity of Methanolic extract.

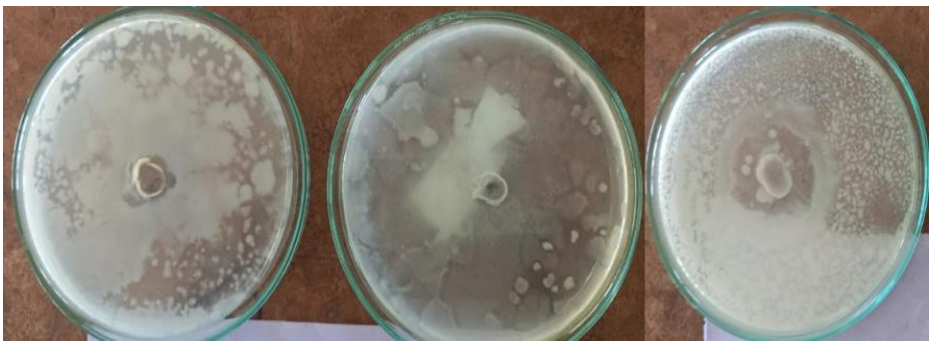

Fig. 6: Antimicrobial activity of Standard drug.

CONCLUSION

The wet leaves of the plant can be subjected to Soxhlation by using methanol as solvent. Then this extraction distilled to get a concentrated mass. Phytochemical investigation was done. The work states that the presence of alkaloids, glycosides, terpenoids, proteins and tannins in the extract of piper betel was responsible for its antibacterial activity. These compounds exhibit a maximum zone of inhibition against Bacillus subtilis. It is interesting to observe the results of high antibacterial effect of methanolic extract. This study gives the way for further studies should be undertaking to elucidate the extract mechanism of action by which extract exert their antibacterial effects. Further studies are need for in-vitro model are required to find out and to establish effectiveness and pharmacological rationale for the use of plant leaves as anthelmintic drug. Biological parameter can be concluded that the methanolic extract of piper betel at 100 µg/ml Shows significant anthelmintic activity.

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