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Pharmacological Evaluation of Polyherbal Extracts of *Amorphophallus paeoniifolius, Citrus sinensis* and *Plumbago zeylantia* for Anti-Microbial and Anthelmintic Activity

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Abstract
Recently there has been a lot of attention focused on producing medicines and products that are natural. Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several species of microorganisms. The worldwide emergence of Escherichia coli, Klebsiella pneumoniae, Haemophilus and many other ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of E. coli and K. pneumoniae are widely distributed in hospitals and are increasingly being isolated from community acquired infections. Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries. This research suggests the aid of polyherbal combination of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia* which may enhance the potency of the medicinal plants even in case when they use alone individually.

Keywords
Amorphophallus Paeoniifolius, Citrus Sinensis, Plumbago Zeylantia, Antimicrobial Activity, Anthelmintic activity.

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INTRODUCTION
For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. During the last few decades, the global interest in the study of various medicinal plants has increased rapidly due to their antibacterial and antioxidant activities, low toxicity and the potential to be a cheaper alternative to costly synthetic drugs. The determination of antibacterial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms. The uses of antibiotics are widespread in clinical medicine, agriculture, and veterinary promote the development of antibiotic resistances among

infectious microbial strains and eventually reflects a very serious problem in the treatment of pathogenic microbes, this has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages. Natural products are typically secondary metabolites, produced by plants and microorganisms in response to external stimuli such as nutritional changes. They are widely used in the pharmaceutical industry for their remarkable structural diversity and range of pharmacological activities. The present study was aimed to determine the potential antimicrobial and anthelmintic activities of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia.*

MATERIAL AND METHODS

Amorphophallus paeoniifolius (*Dennst*.) *Nicolson* (Araceae) is a commonly available tuber in South India, widely used in folk medicine for treatment of acute rheumatism, tumors, lung swelling, asthma, vomiting, and abdominal pain. So far, no attempts have been made to evaluate the chemical composition and medicinal properties of *A. paeoniifolius.* Hence, the present study was performed to investigate the antifungal potential of ethanol extract of *A. paeoniifolius* using different *in vitro* models.

Citrus sinensis L. is an evergreen tree belonging to the family Rutaceae family. It is native to China but is widely cultivated throughout the world. It currently rank as the most commonly cultivated and commercialized specie of citrus. Citrus fruits, including their by-products, have been reported to possess high medicinal value in addition to their economic values. The fresh fruits are consumed on a large scale and large quantities are processed to produce juice. The wastes produced, after consumption and juice extraction, such as the peels, pulps and seeds are a potential source of valuable byproducts. The oil obtained from the seeds, flowers, fruits and rinds of different species of Citrus also find wide applications in the toiletry, confectionary, and perfumery industry. The oils extracted from the peels and seeds of C. sinensis have also been reported to possess different type of activities ranging from insecticidal activity, to antimicrobial activity against a wide range of microbial organisms, hence this study was designed to evaluate and validate the activity of C. sinensis seed oil through an in vitro antifungal assay.

Plumbago zeylanica L. is a multipurpose medicinal herb of family Plumbaginaceae. A native of South Asia, the species is distributed throughout most of the tropics and subtropics. In India P. zeylanica commands an important place among medicinal herbs in India since ancient times. Ayurveda, the Indian indigenous system of medicine dating back to the Vedic ages (1500-8000 BC), has described chitraka as tumor-negating and anti-dyspepsic. Plumbagin has shown antibacterial activity against both gram-positive (e.g. Staphylococcus, Streptococcus, Pneumonococcus sp.) and gram-negative (e.g. Salmonella, Neisseria) bacteria. It is also active against certain yeasts and fungi (Candida, Trichophyton, Epidermophyton and Microsporum spp.) and protozoa (Leishmania).

Preparation of extracts

Air dried coarsely powdered plant materials *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia* were extracted with ethanol (95%) using soxhlet apparatus for 4-5 hrs. All the extracts were concentrated at low pressure by rotary flash evaporator and finally air-dried.

Antimicrobial activity

The different solvent soluble fractions were subjected to antibacterial evaluation against four bacterial strains Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhi (S. typhi), Staphylococcus aureus (S. aureus). The media along with petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121°C and 15 psi pressure. The media was poured into Petri dishes under aseptic condition. The stock solutions of corresponding fractions were prepared in dimethyl sulfoxide (DMSO). Bacterial strains were spread on the solidified agar media, then 7 mm wells were punched in the agar media by using sterile metallic borer. Stock solutions of crude extract and fractions in DMSO at concentration of 20 mg/ml were prepared and 200 µl from each stock solution was added into respective wells. The petri dishes were incubated at 37oC for 24 hours and control wells containing antibiotic (Levofloxacin), which is a positive control, was also run side by side. After 24 hours antibacterial activities were measured as diameter of the zones of inhibition and compared with the zone of inhibition of control (Levofloxacin).

Anthelmintic activity

The activity was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug. The assay was also performed on the aquarium worm, *Tubifex tubifex*, because they belong to same group of Annelida. Briefly, 20 ml formulations containing two different

concentrations of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia* were prepared and six earthworms (same size) and approximately 5 g of *Tubifex* worms were placed in it. Both the test solution and standard drug solution were freshly prepared and 'time for paralysis' was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The 'time for death' of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°. A maximum time period of 120 min was ascertained for the paralyzing as well as death time of *Pheretima posthuma* and *Tubifex tubifex* worms. Piperazine citrate (10 mg/ml) was used as reference standard with distilled water as the vehicle control. All experiments were repeated thrice. The mean and SEM were analyzed statistically by ANOVA followed by Dunnett's test, $P<0.05$ being considered as significant.

RESULTS

Table 1: Anti-microbial activity of leaf extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia*.

Treatment	Frictions	Zone of Inhibition (mm)			
		E. Coli	*P aeruginosa*	*S. typhi*	*S.aureus*
DMSO		0	0	0	0
Amorphophallus Paeoniifolius	Ethyl acetate	14.2 ± 1.12	15.31 ± 0.76	14.18 ± 1.41	13.2± 1.53
Amorphophallus Paeoniifolius	Butanol	15.62 ± 0.54	16.47 ± 0.59	14.54 ± 1.76	14.62± 0.13
Citrus Sinensis	Ethyl acetate	16.26 ± 0.72	15.54 ± 1.35	16.04 ± 0.18	15.32± 0.37
Citrus Sinensis	Butanol	17.52 ± 0.26	17.31 ± 0.51	17.53 ± 0.96	16.62± 0.84
Plumbago Zeylantia	Ethyl acetate	14.26 ± 0.31	15.94 ± 1.58	14.91 ± 0.41	14.52± 1.03
Plumbago Zeylantia	Butanol	15.42 ± 0.64	15.91 ± 0.16	15.13 ± 0.68	15.02± 0.18
Polyherbal Extract	Ethyl acetate	20.28 ± 0.23	19.48 ± 0.19	19.35 ± 0.34	20.71± 0.33
Polyherbal Extract	Butanol	21.42 ± 0.72	21.31 ± 0.46	22.40 ± 1.67	21.27± 1.36
Levofloxacin		23.4±0.16	24.1±1.02	24.16±1.13	23.8±0.28

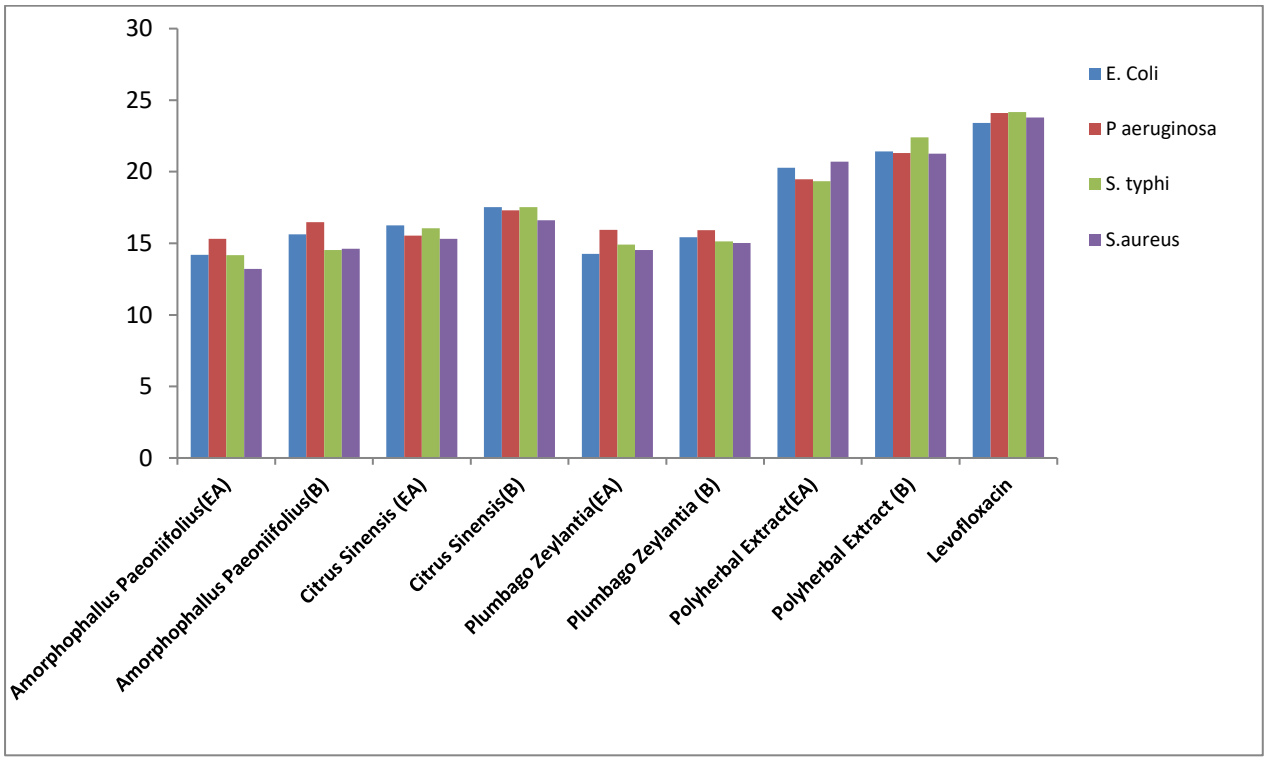

Figure 1: Anti-microbial activity of leaf extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia*.

Table 2: Anthelmintic activity of leaf extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia*.

Treatment	Concentration (mg/ml)	*Pheretima Posthuma*	
		Paralyzing time (min)	Death time (min)
Distilled Water		0	0
Amorphophallus Paeoniifolius	5	60.54 ± 0.34	80.13 ± 0.62
	10	31.12 ± 0.86	63.54 ± 0.45
Citrus Sinensis	5	56.18 ± 0.32	74.15 ± 0.61
	10	34.46 ± 0.84	51.14 ± 0.93
Plumbago Zeylantia	5	53.62 ± 0.16	76.52 ± 0.94
	10	38.37 ± 0.12	55.06 ± 0.45
Polyherbal Extract	5	45.22 ± 0.72	51.54 ± 0.62
	10	38.48 ± 0.93	47.26 ± 0.78
Piperazine Citrate	10	25.14 ± 0.39	41.82 ± 0.14

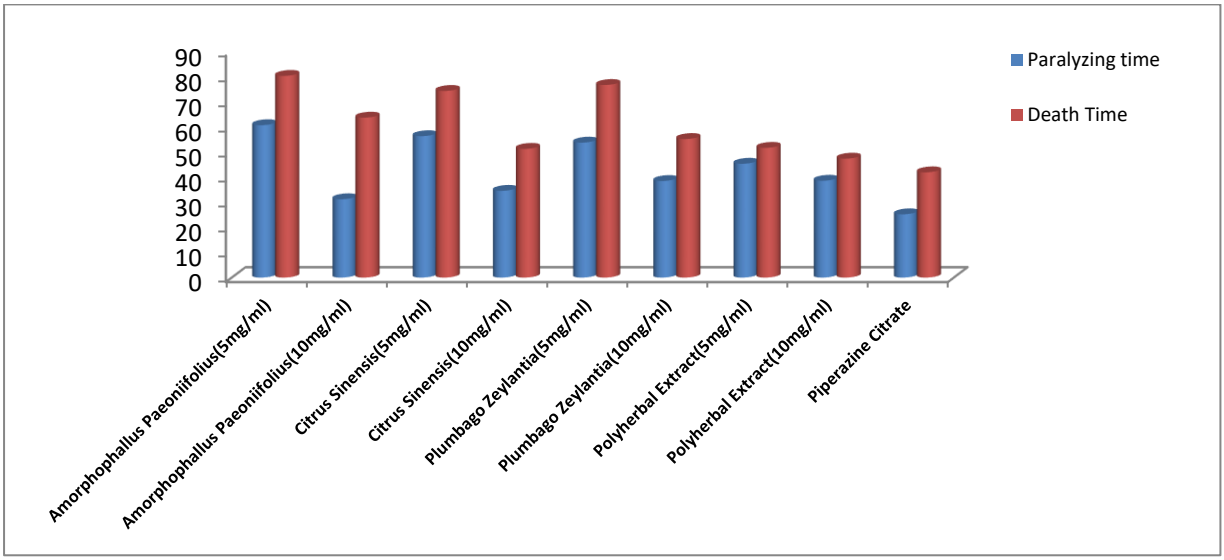

Figure 2: Anthelmintic activity of leaf extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia*.

DISCUSSION

As per the research study, plant extracts with different solvents shows a potent antimicrobial activity as the zone of inhibition increases when the polyherbal extract was introduced. Levofloxacin as standard drug was compared with the polyherbal extract for antimicrobial activity and was found to be effective than the extracts when used alone.

From the observations made, a dose dependent paralytic effect much earlier and the time of death was observed (Table 2). Although, plant extracts showed anthelmintic activity in a dose-dependent manner but the polyherbal extract appeared to be more effective in killing of worms. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate.

CONCLUSION

The Polyherbal Extracts of *Amorphophallus Paeoniifolius, Citrus Sinensis* and *Plumbago Zeylantia* for Antimicrobial and anthelmintic Activity was evaluated and was found to be very potent when they were given in combination than individual extracts given alone.

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