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Anti-*Helicobacter Pylori* and Cytotoxic activity of detoxified root of *Plumbago auriculata*, *Plumbago indica* and *Plumbago zeylanica*

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Abstract

Anti-*Helicobacter pylori* and cytotoxic activity of detoxified root ethanol extract of *Plumbago auriculata*, *Plumbago indica* and *Plumbago zeylanica*. These three Plumbaginales root were detoxified with lime water and prepared the ethanol extract. Ethanol extract of these plants are possible activity against *H. pylori* and cytotoxicity activity with MTT assay in HGE-17 cell lines. These three plants ethanol extract (50-250 µg/ml) have dose dependent cytotoxicity activity in HGE-17 cell lines. Zone of inhibition test of these Plumbaginales plants ethanol extract against H. pylori have significant activity. *Plumbago indica* (10 mg) have more activity compared to other two plants. Three Plumbaginales detoxified plants root have cytotoxicity in HGE-17 cell lines and antibacterial activity in *H. pylori*. Based on our results these three detoxified plants root are used for *H. pylori* induced gastric ulcer.

Keywords: *Plumbago auriculata*, *Plumbago indica*, *Plumbago zeylanica*, Ulcer, *H. pylori*.

Introduction

Peptic ulcer disease (PUD) is one of the most common, chronic gastrointestinal disorders in modern era. It has become a common global health problem affecting a large number of peoples worldwide and also still a major cause of morbidity and mortality. An estimated 15,000 deaths occur each year as a consequence of PUD in India. A report of the Indian Council of Medical Research on the epidemiology of peptic ulcer in India showed that the overall incidence of the disease ranged up to 7% in the age group of 15 years and above in the selected urban population.[1] This situation forced to found a new medicine for peptic ulcer, especially *H. pylori* induced ulcers.

Reviews of the drugs derived from herbal plants which are more commonly used in the world for treatment of peptic ulcer and *H. pylori*, which can say as anti-ulcer activity and having gastroprotective effects; it is reported that the *Plumbago indica* is having antibacterial activity and Mullu Kuruma tribe of Wayanad district in Kerala uses ethnomedicines like *P. indica* as antibacterial Herb.[2, 3] *Plumbago zeylanica* is kill intestinal parasites[4] and it is also used as antibacterial agent in Panchakarma Ayurvedic Therapy[5] and also in Taiwanese folk medicine for anti-Helicobacter activity.[6] The tribal's of Maharashtra are having the habit of consuming the root juice or extract of *Plumbago auriculata* for gastric acidity before each meal for a weak.[7] The investigations based on the enzymatic screening with the root extracts of *P. Indica*,

P. zeylanica, and *P. auriculata* has shown the presence of some powerful enzymes in the root of Plumbago species which act as gastro-intestinal flora normaliser. The *P. zeylanica* flowers showed greater effect on digestive stimulus activity than the other Plumbago species.[8]

Ethnomedicinal plants to fight neoplastic diseases by *P. zeylanica* is used for treating diarrhoea, dysentery, piles and peptic ulcers, which can later develops to neoplasm. These reviews already reported that these selected plants of Plumbago species may protect the gastric mucosa may cure gastric ulcers.[9] Hence we assess the significance of these observations to give a scientific explanation to the anti- *H. pylori* and cytotoxicity activity of *P. indica*, *P. zeylanica* and *P. auriculata* pants in Plumbaginacea.

Materials and Methods

Detoxification and ethanol extract preparation

Roots of *P. auriculata*, *P. indica* and *P. zeylanica* (1 Kg) were dried, coarsely powdered and soaked in lime water separately. The lime water was frequently changed till the red colouration of lime water disappears. Then the roots are again dried, finely powdered and extracted using soxhlet apparatus for 6 hours using ethanol as solvent. The extract was concentrated and dried at 40 degree C under low pressure using rotary vacuum evaporator. The percentage yield of *P. auriculata*, *P. indica* and *P. zeylanica* ethanol extract were 0.78, 1.5 and 1.37 respectively.

Microculture tetrazolium (MTT) Assay

Cell viability was assessed by MTT assay (Micro culture tetrazolium/formazan assay) in the presence and absence of different concentrations of the plants extract. The cells were seeded in 96-well plates. Four wells for each concentration were seeded and triplicate plates were used the cell line. Then, the cells were incubated at 37° C. After 24 h the medium was replaced by fresh medium containing different concentrations of the plants extract. Then, the medium was changed by fresh medium containing MTT with a final concentration of 0.5 mg/ml (after 24 h). The cells were incubated for another 4 h in a humidified atmosphere at 37° C and after that the medium containing MTT was removed and remaining MTT formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm. IC_{50} was defined as the concentration of the extract that produced a 50% inhibition in cell viability relative to the negative control which was wells exposed to the solvent without any extract.[10]

Inhibitory-zone testing

The extracts were used for inhibitory-zone testing with concentration of 100 µg/ml. Anti- *H. pylori* inhibitory-zone testing consisted of three plants ethanol extract against *H. pylori*. A volume of 0.1-ml of each tested *H. pylori* suspension was spread onto a Columbia agar plate. Wells were punched (5 mm) on the plates and the extract [ethanol as solvent] was individually incorporated into the wells (10 mg / well). Ethanol was used as control. The plates were diffused at 4°C for 2 h and subsequently incubated in a microaerophilic jar system (5% O_2 and 10% CO_2) at 37° C for 72 h. The clear zone around each well was observed and its diameter was examined.[6]

Statistical analysis

The values are expressed as mean ± SD and the significance between different groups were determined by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison has been done, with a P value of cytotoxicity study is 0.0044, F value of 6.485 and zone of inhibition study is P<0.0001, F value of 284.9. Probit values were calculated using probit table and IC_{50} of each sample were calculated, using probit value.

Results

Table 1 showed the percentage cytotoxicity of *P. auriculata*, *P. indica* and *P. zeylanica* plants ethanol extract (50, 100, 150, 200 and 250 µg/ml) was done in MTT assay using HGE-17 Cell lines. *P. indica* have higher cytotoxicity activity and dose dependant manner compare to *P. zeylanica* and *P. auriculata*. Concentration 250 µg/ml of *P. indica* and *P. zeylanica* has same percentage of cytotoxicity activity. Table 2 showed IC_{50} value of three plants ethanol extract, values were calculated as percentage of cytotoxicity with probit table method. IC_{50} value of *P. indicia* have 178.29 µg/ml, it is lesser to other two plants *P. zeylanica* have 199.94 µg/ml and *P. auriculata* have 278.59 µg/ml.

Table 3 showed the effect of *P. auriculata*, *P. indica* and *P. zeylanica* ethanol extract inhibitory zone test against *H. pylori* organism. The agar diffusion method was used to study the anti- *H. pylori* activity of the plants ethanol extract. Among the three plants, *P. auriculata* extract had the lowest zone of inhibition against the *H. pylori* strains (1.17 cm), which was followed, in ascending order, by *P. zeylanica* and *P. indica,* (1.35 and 2.17 cm). Tukey's multiple comparison test show that the *P. indica* shows high significance when compared to the solvent which is

having zone of inhibition of 0.47 cm; with a P<0.0001, F value of 284.9.

Table 1: Cytotoxicity effect of *P. auriculata, P. indica* and *P. zeylanica* ethanol extract in MTT assay using HGE-17 Cell lines

Concentration of extract (µg/ml)	% Cytotoxicity		
	P. indica	***P. zeylanica***	***P. auriculata***
50	14.75 ± 0.32	17.99 ± 0.11	12.74 ± 0.37
100	37.54 ± 1.25	30.84 ± 0.50	18.77 ± 0.16
150	43.80 ± 0.30	37.84 ± 0.18	24.77 ± 0.35
200	54.97 ± 0.50	47.93 ± 0.44	44.80 ± 0.21
250	57.77 ± 0.55	57.99 ± 0.30	51.73 ± 0.65

Values are Mean ± SD of three separate experiments performed in triplicates for each concentration.

Table 2: IC_{50} value of *P. auriculata, P. indica* and *P. zeylanica* ethanol extract in MTT assay using HGE-17 Cell lines

Extracts	IC_{50} (µg/ml)
P. indica	178.29
P. zeylanica	199.94
P. auriculata	278.59

Table 3: Effect of *P. auriculata, P. indica* and *P. zeylanica* ethanol extract inhibitory zone test against *H. pylori organism*

Extracts	Zone of inhibition (cm)
Solvent (Ethanol)	0.47 ± 0.06
P. indica (10 mg)	2.17 ± 0.12
P. zeylanica (10 mg)	1.35 ± 0.07
P.auriculata (10 mg)	1.17 ± 0.02

The values are Mean ± SD of the zone of inhibition (cm) against *H. pylori* of three separate experiments performed in triplicates for each plant extract.

Discussion

Plumbagin at low doses gives stimulant action on nerves but at high doses it causes irritation to skin and is highly toxic, which leads to paralysis and ultimately death. Plumbagin is mainly allelopathic and contact irritant.[11] Many reports reveal that the administration of Plumbagin is associated with severe acute toxicity.[12] It is a highly toxic and acts like a spindle poison by inhibiting cell mitosis at low concentrations. At higher concentrations it exhibits radiomimetic, nucleotoxic (arrest of cell proliferation and decrease in mitotic index, with evidence of chromosomal aberrations) and cytotoxic effects.[13] Our preliminary result of the estimation plumbagin concentration in non detoxified *P. indica* is higher than the other Plumbaginales. Unnikrishnan *et al.*, reported that the purification of Plumbaginales using limewater, excess of Plumbagin oozes out into the lime water.[14] Hence lime water treatment is adopted for the detoxification of Plumbago species.

The result that the non-cancerous cell line HGE-17 is not extensively inhibited by the detoxified plants ethanol extract indicates that the growth inhibitory effects of cell lines are not the result of a general toxicity of the plants. Different concentrations of three plants ethanol extract were tested for cytotoxic activity. Within 48 hours of test period – the time that the extract was in contact with the cells the effect on cell cycle may not have been so pronounced as with longer periods. IC_{50} indicated that for the non-cancerous human cell line HGE-17, the growth was inhibited by detoxified ethanol extract of three Plumbaginales. This preliminary screening model helps to develop new antineoplastic agents. The present study reveals that Plumbaginales are having cytotoxic activity even in the absence of Plumbagin.

Inhibitory-zone testing is the primary method for evaluation of the susceptibility of the test samples against specific microorganisms. Three plants ethanol extract significantly inhibit growth of *H. pylori*, in descending order *P. indica, P. zeylanica* and *P. auriculata*, which offers advantages, i.e., greater convenience and reduced cost for the treatment of *H. pylori* infections in comparison to proton-pump inhibitors or H_2-blockers treatments. With a high prevalence with antimicrobial resistance, herbal therapy has to be implemented for the termination of *H. pylori* attacks. It is clear that three Plambaginale plants ethanol extract having measurable growth inhibitory effect in the cell proliferation and also in antibacterial activity, which will be a future prospect as antiulcer as well as anticancer activity.

Conclusion

Based in our results, the three detoxified *P. indica, P. zeylanica* and *P.auriculata* can be considered as a source of compounds with anti-*H. pylori and cytotoxic* activity. Further we research to isolate pure compounds from different extracts as well as establishing the toxicities of extracts of the Plumbago species, may offer medicinal uses for the indigenous population (at little cost) or offer a structure (of a pure compound) for pharmaceutical development. If extracts would be used on large scale for medicinal purposes, it is of utmost importance that cultivation and conservation of this plant accompany the use for medicinal purposes.

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