IN VITRO ANTI-AMOEBIC ACTIVITY OF STEM BARK OF AILANTHUS EXCELSA, ROXB (SIMAROUBACEAE)

G. Prakash Yoganandam[1]*, K. Periyanayagam[2], K.Ilango[3], Diptanu Biswas[1], R. Gowri[1].

1. Department of Pharmacognosy,
SRM College of Pharmacy, SRM University, Kattankulathur-603203.
Kancheepuram District, Tamil Nadu, India.

2. Madurai Medical College, Madurai – 625020,
Tamilnadu, India.

3.Department of Pharm.Chemistry, SRM College of Pharmacy, SRM University,
Kattankulathur-603203.
Kancheepuram District, Tamil Nadu, India.

Summary

Ailanthus excelsa has the traditional value of treating diarrhoea and dysentery. The presence of quassinoids as active constituents in *Ailanthus excelsa* strengthens the fact. The aqueous, petroleum ether and defatted ethanolic extracts (Quassinoid fraction) of stem bark of *Ailanthus excelsa* was tested against the laboratory cultured *Entamoeba histolytica* for its anti-amoebic action using metronidazole as standard drug. The EC_{50} value for aqueous, petroleum ether and defatted ethanolic extracts (Quassinoid fraction) were 195, 185 and 150 µg/ml against *E. histolytica* respectively. The isolation of active principle responsible for the activity may give a potent, anti amoebic drug molecule with lesser side effects. This study provides validity of the traditional claim.

Short Title: Anti-amoebic activity of Ailanthus excelsa, Roxb.Simaroubaceae.

Key words: *Ailanthus excelsa*, Simaroubaceae, Antiamoebic activity, *Entamoeba histolytica.*

***Corresponding author**
G. Prakash Yoganandam, M.Pharm,
Department of Pharmacognosy, SRM College of Pharmacy, SRM University, Kattankulathur-603203.
Kancheepuram District, Tamil Nadu, India.

Introduction

Amoebiasis was defined by WHO as "a condition in which a patient is harboring the organism *E .histolytica* in the bowel". Amoebiasis is the infection, which remains a significant cause of morbidity and mortality worldwide[1].

An estimated 12% of the world's population harbor *E. histolytica* and as a result amoebic dysentery is common in the tropics and subtropics if left untreated, this condition may lead to amoebic liver disease and other serious complications[2].

The nitroimidazoles, metronidazole is a highly effective amoebicide and is considered by many clinicians to be the drug of choice for treating acute amoebiasis. However, metronidazole has mutagenic effects in bacteria and is carcinogenic to rodents. The drug is relatively ineffective against asymptomatic infections in the intestinal lumen ("cyst-passers") [3]and adverse effects, especially severe nausea, and interactions with alcohol may reduce the level of patient compliance. In addition, amoebae may develop resistance to metronidozole. Clearly, there is a need for alternative antiamoebic agents[4].

Therefore the search of new compounds with amoebicidal activity is urgent and important. *Ailanthus excelsa* (Family: Simaroubaceae) is a large, deciduous tree, up to 24 m in height and 2.5 m in girth, indigenous to Central and Southern India. It is widely used in the Indian system of medicine especially in the treatment of diarrhoea and dysentery.[5]

The bark is bitter, astringent, febrifuge, anthelmintic and used in diarrhoea and dysentery. The bark is good substitute for kurchi bark (*Holarrhena antidysenterica, Wall*) and is used in indigenous veterinary practice[6, 7.] Presence of quassinoids in *Ailanthus excelsa* was reported by researchers[8] .Pronounced amoebicidal activity associated with quassinoids and its traditional use in diarrhoea and dysentery were the reason for the evaluation.

Materials and Methods

Plant Material Collection and authentication

Fresh stem bark of *Ailanthus excelsa* was collected from Alagar koil hills of Madurai district, Tamil Nadu, India. Care was taken to collect only the healthy barks then it was authentified by Prof.S.Stephen, M.Sc, Ph.D, of the department of botany, The American College, Madurai, India. Voucher specimen number **(No: AE/MMC/06/0156)** was deposited at the department of Pharmacognosy for further references.

Preparation of extracts

The air dried powder material of the stem bark of *A. excelsa* was extracted with petroleum ether (60-80^{0}C) in a soxhlet apparatus (1.55%) and the aqueous extract was prepared by hot maceration processes[8] (5.6%). The defatted ethanolic extract (quassinoid fraction) was prepared by defat the stem bark powder with petroleum ether (60-80^{0}C) followed by extraction with absolute alcohol. The alcohol extract on concentration under reduced pressure and refrigeration deposited a solid which contain C_{20} quassinoids (1.28%)[9] .The aqueous extract of *A excelsa* (**AEAE**), petroleum ether extract of *A .excelsa* (**PEAE**) and quassinoids fraction of defatted ethanolic extracts of *A. excelsa* (**QEAE**) at the concentration of 100, 200 and 300 µg/ml were used to test the activity against *E.histolytica*. Metronidazole was used in the same concentrations as a standard drug. DMSO was used as vehicle control.

Invitro culture of *Entamoeba histolytica*

(i) Preparation of culture medium[10, 11]

The culture was done in the biphasic polyxenic medium, which constituted the egg slope and an overlay solution (Locke's solution). Egg slope was prepared by mixing 270 ml of fresh egg suspension with 75 ml of Locke's solution [NaCl 9.0g, $CaCl_2$ 0.3g, KCl 0.4g,

$NaHCO_3$ 9.0g, Glucose 2.5g and distilled water 1000 ml]. The mixture (2.5 ml) was dispensed aseptically into sterile culture tubes and insipitated in a slanted position at 70^oC for 30 minutes. The overlay solution was obtained by mixing 8 parts of sterilized Locke's solution with 1 parts of inactivated human serum. To complete the medium, 5ml of overlay solution was added to each tube containing egg slope.

(ii) Culture of Parasites[12]

Just before the time to sow, a loopfull of sterilized rice starch (1mg) was added to the medium. Then a small quantity of stool (collected from positive carriers) was inoculated into the culture medium and incubated at 37^0 C for 48 hrs. After this time, the culture fluid in the tube was mixed and then examined under microscope for amoebal growth. In order to renew the culture medium, the culture tubes were chilled on ice for 5 minutes and the upper phase (around 4 ml) was discarded. The sedimented part containing the parasite was mixed and transferred to a fresh tube containing the culture medium and rice starch.

Measurement of amoebicidal activities of plant extracts[13]

To 5ml of the medium in the tubes, 1ml of each concentration of plant extracts (T_e) and 1 ml of the amoebic inoculum's containing around $10x10^6$ trophozoites were added. Each test is performed in duplicate and one control (T_c) using DMSO and the standard drug metronidazole (T_S) at the same concentrations were used.

All the tubes were incubated at 37^0C for 48 hours after this time 1ml of medium was taken off from each tube for the viability count using trypan blue dye exclusion technique by haemocytometer. The percentage mortality was calculated by using the following formula. The EC_{50} was calculated by regression analysis.

$$\% \text{ Mortality due to extract} = \frac{\text{No. of deadcells in } (T_e) - \text{No.of dead cells in } (T_d)}{\text{Total number of cells (10)}} \times 100$$

Statistical analysis

The percentage mortality was calculated for each concentration. The plot of percentage mortality against log concentration was made and the best fit line is determined by regression analysis. Regression analysis was used to calculate the effective dose 50(EC_{50}), defind as the dosage of drug to produce 50% mortality. The level of significance was determined by one-way ANOVA. A confident level of 95% ($p<0.05$) was considered as statistically significant.

Results and Discussion

It was observed that the quassinoid fraction (QEAE) from defatted ethanolic extracts of *A. excelsa* has significant antiamoebic activity and comparable to the standard drug metronidazole. The aqueous and petroleum ether extracts also having antiamoebic activity comparable to QEAE (Table 1, Fig 1) The EC_{50} values for QEAE, AEAE and PEAE were 150, 185 and 195 µg/ ml respectively. The decreasing order of antiamoebic activity was QEAE>AEAE> PEAE.

The quassinoids (or) simaroubolides are a group of terpenoid related compounds isolated from a variety of plants in the simaroubaceae. Many of plants have folk medicine history particularly for antiamoebic activity and a number of isolated quassionids are currently of interest for their antitumour properties[12].

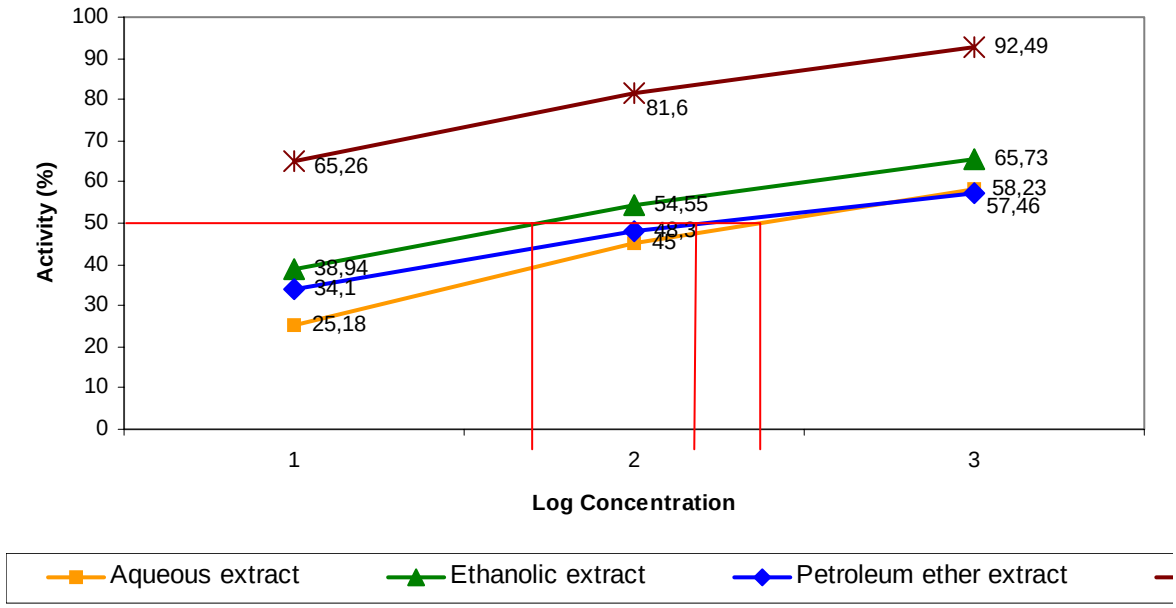
The four quassinoids from *Simarouba amara* had significant antiamoebic activity, with ailanthinone being twice as potent as the others. In contrast glaucarubol in *S.glauca* was inactive against amoebae. Quassinoid itself, which is constituent of *Qussia amara* and *Picrasma excelsa*, was also inactive against amoebae. From *Brucea Javanica* and *Simarouba species*, Bruceantin was the most active quassinoid and yadanzioside F was the least active quassinoid against *E. histolytica*.

Table 1

Effect of Aqueous, Ethanolic and Petroleum ether extract of *A.excelsa* against *E.histolytica* (*Invitro*)

Drug	Concentration (µg/ml)	Number of Dead Cells ($X10^6$)	% Mortality due to extract $\frac{T_e - T_d}{Total\ No\ of\ Cells\ (10)}\ x\ 100$	Corrected % Mortality using Abbot's formula
Aqueous extract	100	2	20	20
	100	2	20	
	200	4	40	45
	200	5	50	
	300	7	70	70
	300	7	70	
Ethanolic extract	100	3	30	35
	100	4	40	
	200	5	50	55
	200	6	60	
	300	7	70	75
	300	8	80	
Petroleum ether extract	100	3	30	30
	100	3	30	
	200	4	40	50
	200	6	60	
	300	6	60	65
	300	7	70	
Standard drug (metronidazole)	100	6	60	60
	100	6	60	
	200	8	80	85
	200	9	90	
	300	10	100	100
	300	10	100	
Control (DMSO)	50µl	-	-	-
	50 µl	-	-	-

Fig.1
REGRESSION LINE FOR ANTI AMOEBIC ACTIVITY OF VARIOUS EXTRACT OF A. EXCELSA AGAINST E. HISTOLYTICA



Some quassinoids are known to inhibit protein synthesis in mammalian cells however their mode of action against *E. histolytica* remains to be investigated [4].

The anti amoebic activity of stem bark of *A.excelsa* may be attributed to the phytocostituents present in the plant and the quicker process of anti amoebic could be functions of either the individual or the additive effects of the phytoconstituents. The present study has demonstrated that the ethanolic extracts (Quassinoids fractions) of A.excelsa stem bark has properties that render it capable of promoting accelerated anti-amoebic activity compared with control. Further phytochemical studies are in process to isolate, characterize and identify the specific active compounds in this plant responsible for anti-amoebic activity.

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