Original article:

Comperative phyto toxicological and anti inflammatory effects of leaves extracts of *holoptelea integrifolia*

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Abstract

Objective: Plants play valuable role in the new drug discovery and significantly used to treat different diseases. Nowadays scientists are investigating the therapeutically active phytochemical constituents that are safe and producing lesser side effect in comparative to other standard drugs. ***Methods:*** The plant *Holoptelea integrifolia* is medicinally important and this study was carried out to evaluate the anti inflammatory activity of aqueous extract of the leaves of *Holoptelea integrifolia* in male albino rats wistar stain treated with acetic acid to induced paw edema. ***Results:*** Result indicated the significant anti-inflammatory activity while compared with standard drug (diclofenac sodium).Brine shrimp bioassay (cytotoxicity), phytotoxicity, insecticidal and enzyme inhibition activity was performed in different extracts of the leaves of *H. integrifolia.* Results of brime shrimp bioassay indicating positive lethality at high dose in BuOH and H_2O only. While the results of phytotoxicity in all crude extracts displayed mild phytotoxicity(46.3 µg/ml) in high concentrations (1000 µg/ml) except H2O extract showed no phytotoxicity. Result of insecticidal activity revealed that BuOH extract were found more effective against *Rhyzoperthadominica,* the EtOH extract expressed major while EtOAC extract showed mild activity against *Callosobruchusanalis.* Aqueous extract possessed no insecticidal activity. ***Conclusion:*** Results of Urease inhibition activity suggested that EtOAC and BuOH extracts of this plant expressed no activity while EtOH and H_2O possessed mild inhibiting activity.

Keywords: *Holoptelea integrifolia*; phytotoxicity; insecticidal activity; enzyme inhibition activity; Anti-inflammatory activity; Brine Shrimp Bioassay

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Introduction

Holoptelea integrifolia (Roxb) Planch. is one of medicinally important traditional plant belongs to family Ulmaceae, commonly known as Indian Elm, indigenous to tropical regions of Asia, also found in different areas of Pakistan especially in Karachi[1-4]. All parts of this plant used to treat inflammation, bacterial infections, diarrhea, tumor, diabetes and wound, etc.[5-10]. Bark and leaves of *H. integrifolia* have been used as bitter, astringent, anthelmintic, acrid, thermogenic, anti-inflammatory, digestive, carminative and laxative to treat various diseases. Seeds and stem bark applied against ringworms externally. Seeds have been used to cure ulcers and as body deodorizer[4,11-14]. Leaves of *H. integrifolia* possess analgesic, anti inflammation and anti diabetes properties also used for various skin infections, piles and gastrointestinal diseases[15,16]. Alkaloids, tannins, cardiac glycosides, saponin glycosides, cyanogenetic glycoside and anthracene derivatives.

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Hexacosanol, octacosanol, β-sitosterol and β-amyrin have been obtained in alcoholic extract of leaves of *H.integrifolia*[17-19]. This research has been conducted for the anti-inflammatory activity with aqueous extract of plant *H. integrifolia* in compression to standard drug (Diclofenac sodium)[20]. While different extracts of *H. integrifolia* were selected to evaluate the cytotoxicity, phytotoxicity, insecticidal and enzyme inhibition activities of this medicinally valuable plant.

Material and methods

***Plant collection*;** The fresh leaves of *H. integrifolia* were collected from the Faculty of Pharmacy, University of Karachi, Pakistan and the plant material was dried (500 g), chopped and macerates with methanol (MeOH) for 15 days after identification. Solvent was evaporated at reduced pressure and controlled temperature to obtained MeOH extract. This extract was subjected for step by step extraction with *n*-hexane, ethanol (EtOH), ethyl acetate (EtOAC), butanal (BuOH) and H_2O then concentrated in rotary evaporator to obtained different fractions. Brine shrimp bioassay, phytotoxicity[21], insecticidal activity and enzyme inhibition activity (indophenol method)[22] were performed. For the determination of insecticide activity and toxicity different methods are being used on various surfaces (Insecticide impregnated dust on grain, direct spray on grain, impregnated filter paper test)[23-25]. Anti-inflammatory activity has performed with aqueous leave extract *H. integrifolia*[26-28]. % Inhibition of paw volume = (Control mean – treated mean) / Control mean×100. Ethical approval was taken prior the study.

Results and discussion

Anti-inflammatory activity; Aqueous extract of *H.integrifolia* has significant anti- inflammatory activity. Active constituents of plants play a major role for the discovery of new pharmaceutical products for the treatment of various ailments due to its efficacy and safety[29,30]. Inflammatory diseases are one of the most common causes of different health disorders[31]. Anti-inflammatory studies have been conducted using aqueous extract of the leaves of *H.integrifolia* and outcomes were analyzed in comparison with standard anti-inflammatory drug diclofenac sodium[20,28]. Paw edema was calculated at different time interval using plethysmometer (Table 2). New bould method was used to evaluate percentage inhibition of paw edema (Table 3)[32]. The statistically significant results has been produced in group that is treated with aqueous extract of the leaves of *H. integrifolia* after 3 hours in compression to group that treated with diclofenac sodium that shown % of inhibition after 4 hours. The significant ($P< 0.05$) anti-inflammatory activity of aqueous extract of the leaves of *H. integrifolia* markedly reduced the paw edema in compression to standard drug i.e. Diclofenac sodium against acetic acid induced paw edema in albino rats at a dose of 250mg/kg /body weight and outcomes have been mentioned in Fig. 1 and 2 (Table 1).

Table 1: Anti-inflammatory Activity of the Aqueous Extract of *H.integrifolia*

Group	Mean increase in Paw volume with SEM						
	0hr	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**
I(NC)	2.90±0.19	2.90±0.19	2.90±0.19	2.90±0.19	2.90±0.19	2.90±0.19	2.90±0.19
II (AA)	2.79±0.19	3.01±0.22	3.64±0.18	4.29±0.14	4.61±0.16	4.94±0.17	5.19±0.20
III (DS)	2.52±0.12	2.83±0.13	3.60±0.14	4.15±0.14	3.95±0.09*	3.72±0.13*	3.60±0.22*
IV(AEH)	2.38±0.04	2.84±0.05	3.16±0.04	3.6±0.05*	3.39±0.04*	3.11±0.06*	2.84±0.04*

(Where: NC= Normal control; AA= Acetic acid (positive control); DS= Diclofenac sodium, AEH= Aqueous extract of *Holoptelea integrifolia* (Roxb) Planch. ; * = significant value *P* values < 0.05 as compared with acetic acid (positive control))

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA). *P* values < 0.05 were considered significant.

Percentage Inhibition of Paw edema (on 5th day); the results of percentage inhibition of paw edema with aqueous extract of *H.integrifolia* and standard drug (diclofenac sodium) compared with acetic acid (Table 2).

Table 2: Percentage Inhibition of Paw Edema.

Percentage inhibition (hr)	0	1	2	3	4	5	6
DS	9.719	5.924	1.052	3.297	14.409	24.713	30.584
AEH	14.728	5.481	13.226	16.214	26.435	37.053	45.250

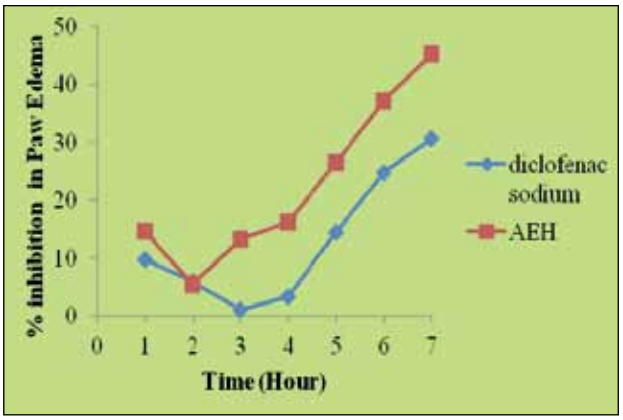

Fig. 1. Comparison of inhibitory effects in paw volume between aqueous extract and diclofenac sodium

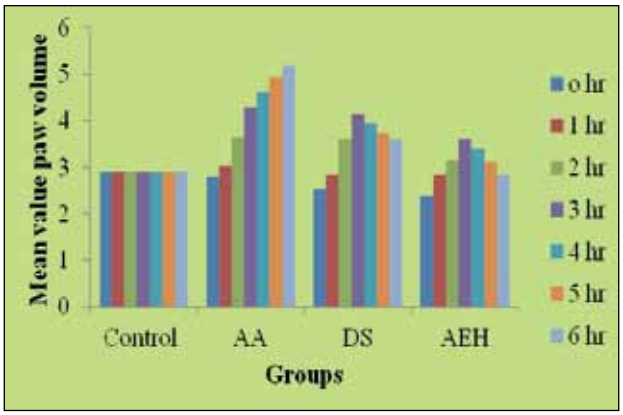

Fig. 2. Means values of paw volume at different time intervals for I, II, III and IV groups

Toxicity Studies

Brine Shrimps Bioassay (LD_{50}); Beside the therapeutic activity of plants and their constituents, some of these are detrimental effects to humans and other living organisms. Shrimp larvae (*Artemiasolina*) are susceptible to various harmful constituents and are used to evaluate the toxicity. Different extracts (EtOH, EtOAC, BuOH and H_2O) of the plant *H.integrifolia* were subjected for the detremination of LD_{50} activity using brine-shrimps (*Artemiasolani*). Brine shrimp bioassay was conducted in triplicate in three different concentrations i.e. 10, 100, 1000 µg/ml of EtOH, EtOAC, BuOH and H_2O extracts of *H.integrifolia* and Etoposide was selected as control (Table 3,4,5 and 6). Results revealed positive lethality at high dose with LD_{50} 629.5723 and 100,000 µg/ml in BuOH and H_2O extract respectively and compared with the standard drug Etoposide at high concentration dose[33](Finney, 1971). While EtOH and EtOAC extracts were not showed such toxicity against brine shrimps although at high concentration.

Table 3: Brine shrimp toxicity bioassay of EtOH extract of *H. integrifolia*

Dose µg/ml	No. of Shrimps	No. of Survivors				% of Survivors	LD_{50}µg/ml	Std. Drug	LD_{50} µg/l
		1	2	3	Av.				
1000	30	20	22	21	21	70%	-	Etoposide	7.4625
100	30	24	26	25	25	84%	-		
10	30	25	27	26	26	90%	-		

Table 4.Brine shrimp toxicity bioassay of EtOAC extract of *H. integrifolia*

Dose µg/ml	No. of Shrimps	No. of Survivors				% of Survivors	LD_{50}µg/ml	Std. Drug	LD_{50} µg/l
		1	2	3	Av.			Etoposide	7.4625
1000	30	26	27	28	27	90%	-		
100	30	24	25	26	25	84%	-		
10	30	26	27	28	27	90%	-		

Table 5: Brine shrimp toxicity bioassay of n-BuOH extract of *H. integrifolia* (Roxb) Planch.

Dose µg/ml	No. of Shrimps	No. of Survivors				% of Survivors	LD_{50}µg/ml	Std. Drug	LD_{50} µg/l
		1	2	3	Av.				
1000	30	13	15	14	14	47%	629.5726	Etoposide	7.4625
100	30	19	17	18	18	50%	-		
10	30	19	18	20	19	63%	-		

Table 6: Brine shrimp toxicity bioassay of H_2O extract of *H. integrifolia* (Roxb) Planch.

Dose µg/ml	No. of Shrimps	No. of Survivors				% of Survivors	LD_{50}µg/ml	Std. Drug	LD_{50} µg/l
		1	2	3	Av.				
1000	30	11	10	12	11	37%	100.00	Etoposide	7.462
100	30	15	14	16	15	50%	-		
10	30	19	18	20	19	63%	-		

Phytotoxic activity or Lemna bioassay: Growth inhibition and promotion of plants has been screened by Lemna bioassay and results for phytotoxicity of the crude extracts i.e. EtOH, EtOAC and BuOH has been expressed (Table 7,8, 9 and 10). In this study, level of toxicity (0.015 µg/m) was determined against *Lemna minor*using Paraquat as standard drug. All of these crude extracts exhibited limited phytotoxicity i.e. 46.3 µg/ml in high concentrations (1000 µg/ml) while H_2O extract was displayed no phytotoxicity at any concentration.

New herbicidal agents are now demanding to discover due to increasing in herbicide resistant weeds and alteration of synthetic herbicides are limitedly acceptable and effective against the resistant weed biotypes with reference to environmental and health related issues [34,35]. Plants play a significant role to discover new herbicides which might be more convenient, safe, effective and biodegradable, so have fewer threat to the environment and used as substituent to the presently used synthetic agrochemicals. Different plants material, their extracts and / or their isolated active compounds may use as allele chemicals to other plants and be utilize for cultivation [35,36]. The results obtained from the this study showed that *H. integrifolia* leaves extracts of EtOH, EtOAC and BuOH could be useful as natural herbicides in comparison with other related herbal species at high concentration and could be deliberated as a source of bioactive agrochemical.

Table 7.Phytotoxic activity of EtOH extract of *H. integrifolia*

Name of Plant	Conc. of Sample µg/ml	No. of Fronds Survived	% of Growth Regulation	Conc. of St. Drug µg/ml Paraquat
Lemna minor	1000	10	46.3	0.015
	100	12	35.5	
	10	17	8.7	

Table 8. Phytotoxic activity of EtOAC extract of *H. integrifolia*.

Name of Plant	Conc. of Sample µg/ml	No. of Fronds Survived	% of Growth Regulation	Conc. of Standard Drug µg/ml Paraquat
Lemna minor	1000	10	46.3	0.015
	100	15	19.4	
	10	18	3.3	

Number of Fronds in control = 40.

Table 9: Phytotoxic activity of n-BuOH extract of *H. integrifolia*

Name of Plant	**Conc. of Sample µg/ml**	**No. of Fronds Survived**	**% of Growth Regulation**	**Conc. of Standard Drug µg/ml Paraquat**
Lemna minor	1000	10	46.3	0.015
	100	14	24.8	
	10	18	3.3	

Table 10: Phytotoxic activity of H_2O extract of *H. integrifolia*

Name of Plant	Conc. of Sample µg/ml	No. of Fronds Survived	% of Growth Regulation	Conc. of St. Drug µg/ml Paraquat
Lemna minor	1000	15	14	0.015
	100	18	3.3	
	10	19	-2	

Insecticide Activity: The extracts of *H. integrifolia* (EtOH, EtOAC, BuOH and H_2O) are used to conduct insecticidal activity against *Tribolium castaneum, Sitophilusoryzae, Rhyzopertha dominica, Callosbruchus analis*, and *Trogodermagranarium*. All test were carried out in control condition compared with standard drug i.e., Permethrin(Table 11). All four extracts were selected for insecticidal activity by contact method on three stored grain pests i.e. *Triboliumcastaneum, Rhyzoperthadominica*, and *Callosbruchusanalis*. Sample concentration was taken 1019.10 µg/cm^2. Permethrin was employed as standard sample with 235.9 µg/cm^2 concentration and experiment were performed with sample in 1019.10 µg/cm^2 concentration. Some activity against *Rhyzoperthadominica* was observed in EtOH extract, while major activity was shown against *Callosobruchusanalis*. EtOAC extract was found to be only moderately activated against *C. analis* and significant activity was examined in BuOH extract against *Rhyzoperthadominica*; while in similar concentration of both extracts other pests were found ineffective. Aqueous extract not displayed effect against all pests or no insecticidal activity was observed (Table 11).

Table 11: Insecticidal activity of different extracts *H. integrifolia*

S.No.	Name of insects	% Mortality		Sample	Sample	Sample	Sample
		+ve control	-vecontrol	EtOH	EtOAC	BuOH	H_2O
1	***Triboliumcastaneum***	100	-	0	0	0	0
2	***Sitophilusoryzae***	-	-	-	-	-	-
3	***Rhyzoperthadominica***	100	-	40	0	80	0
4	***Callosbruchusanalis***	100	-	100	50	0	0

Concentration of test sample = 1572.7 µg/cm^2, Concentration of standard drug = 235.9 µg/cm^2, +ve control = Permethrin (Copex) standard drug,–ve control = Solvent.

Enzyme inhibition activity: The results showed that crude extracts of *H. integrifolia* (EtOH and H_2O) possessed very weak urease inhibiting activities while EtOAC and BuOH extracts of plant were found ineffective (Table12).

Table 12.In-vitro urease inhibition activity of different crude extracts of ***H. integrifolia***

S.No.	Sample Name	% Inhibition	Activity
1	**Crude extract (EtOH)**	10%	+
2	**Ethyl acetate (EtOAC)**	0%	-
3	**n-Butanolic fraction (BuOH)**	0%	-
4	**Aqueous fraction (H_2O)**	11%	+
Standard drug	**Thio urea**	94%	+++

(-No activity, + Low activity, ++ Moderate, +++ High)

This urea was used as control to perform the enzyme inhibition activity with the leaves extracts of *H. integrifolia* against Jack bean. For the discovery of new antiulcer drugs urease inhibitors have shown effectiveness nowadays [37]. Activity of urease has employed majorly as virulence determinant in the pathogenesis of various diseases in human being and animal health and for agriculture[38,39].So far, designed based on urease inhibition is studied as tool first time, for evaluation of infections originated by using urease producing bacteria. This result indicated that EtOAC and BuOH extracts of this plant showed no activity while EtOH and H_2O exhibited mild inhibiting activity.

Conclusion

This aqueous extract of *Holoptelea integrifolia* has shown potent anti inflammatory activity without any liver and kidney damage. This study showed that *H. integrifolia* leaves extracts of EtOH, EtOAC and BuOH could be useful as natural herbicides in comparison with other related herbal species at high concentration and could be deliberated as a source of bioactive agrochemical.

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