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Antibacterial, Antioxidant and Anti-Inflammatory Potential of the Different Extracts of Holoptelia Integrifolia

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Abstract*: *Medicinal plants are play significant impact in the personal medicine for most of the people all over the world as an alternative live saving medicines and most of their medicinal properties are well known for anticancer activity. The different extracts of Holoptelea integrifolia (H.integrifolia) leaves, stem bark and fruits were studied as a potent natural source of antimicrobial, antioxidant and wound healing potential. This work was carried out to evaluate antimicrobial, antioxidant and anti-inflammatory activity of different extracts of H.integrifolia. The antimicrobial activity of the H.integrifolia ethanolic extract was studied against five fungal and bacterial strains by utilizing the agar well diffusion method and MIC. Among several strain, the ethanolic extract of fruit has shown higher antimicrobial inhibition zone as 9.25-16 mm compare to other two extracts of stem and leaves as 10- 13.25 mm and 6-10.2 mm respectively. The antioxidant activities for different extract were also determined by DPPH free radical assay, Hydroxyl Radical Scavenging and Nitric Oxide Radical Scavenging Activity method. The anti-inflammatory activity also estimated basedon formalin induced paw edema method on Wistar albino rats. The different extracts of leaves, stem bark and fruit parts of Holoptelea integrifolia were estimated for in vivo anti-inflammatory activity against the animal model of female Wistar albino rats. The results of anti-inflammatory activity revealed that the Ethanol extracts showed vital and dose-dependent anti-inflammatory effects. Our findings revealed that aerial parts of H.integrifolia contais potential antimicrobial, antioxidant and anti-inflammatory compounds, which* expose the medicinal potential of the selected plant *could be a significant drug candidates against microbial, oxidative and inflammation-related pathological processes as a future alternative medicine.

Keywords*: *Holoptelea integrifolia, Microbial inhibition concentration, DPPH, Hydroxyl Radical Scavenging, Nitric Oxide Radical Scavenging, Anti-inflammatory.

I. INTRODUCTION

Medicinal plants are getting used for treating diseases based on the bacterial and fungal origin and best results are obtained with a number of them. However, the major scientific investigation and data of the therapeutical impact of these plants are very limited [1]. In recent years the scientific community understand the mechanism of free radical scavenging activity which play critical role of anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs [2,3]. Inflammation and oxidative stress play a vital role in various immunological diseases as primary immune response when ever allergens, injuries, cuts, bacterial and fungal infections raised and also at other harmful stimulus [4]. *H.integrifolia* is a medium greeny wigroius tree of 15-25 m in height and whitish or yellowish grey and cut freshly posses' pleasant smell [5]. *H.integrifolia* is important medicinal plant, is the rich supply of different types of important compounds such as Holoptelin-A Holoptelin-B, Friedlin 2-aminonaphthoquinone, β-sitosterol, stigmasterol and hederagenin where reported from heart wood and bark, while hexacosanol octacosanol and α-amyrin were reported in leaves [6]. The current work was performed to evaluate the antimicrobial, antioxidant and anti-inflammatory activity of different extracts for leaves, stem and fruits of *Holoptelea integrifolia*.

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II. MATERIAL AND METHODS

A. Plant material

The aerial part of fresh stem bark, leaves and fruit of *Holoptelia integrifolia* were collected from the Sheshachalam hills, Andhra Pradesh India. Plant species were authenticated by Prof. C. Sudhakar, S.K University, Anantapuramu, India and voucher specimens, were deposited at the herbarium, Department of Botany, Loyola College (YSRR) Pulivendula. The respective materials were washed with running tap water by 2-3 times thoroughly. Then the materials were air and shadow dried and mechanically crushed into coarse powder of 40 µm sizes by using mixer grinder. Powders were stored separately in air all around tight little covers.

B. Chemicals and Reagents

The entire chemicals were purchased from Tarun Scientifics (Kadapa District, A.P State, India). All the chemical reagents used were of analytical grade.

C. Microorganisms and animals

Healthy adult Albino wistar rats were about 125-250 g and Swiss albino mice were about 20-25 g were collected from the animal facilities of the Rural College of Pharmacy, Devanahalli, Karnataka, India.

were kept in well ventilated house cage and animals had 12 hour day and night schedule with temperature between 23±2°C and relative humidity 75%. During the experimental course period, the animals were kept in large hygienic spacious cages and **it was allowed for free movement according standard laboratory cube pellets and provided drinking water facilites.** The work was carried out after getting clearance of ethical committee from the institutional animal ethical committee. The Bacterial cultures (*Bacillus subtilise, Pseudomonas aeruginosa Micrococcus luteus, Eschericia coli* and *Proteus vulgaris*) and fungal cultures (*Asperigillus niger, Aspergillus flavus, Tricoderma vibriae, Penicillium rubrum* and *Chaetomium globosum*) were got from Microbial collection center for Type Culture(MTCC), Chandigarh, India. They cultures were grown at 4°C on nutrient agar medium.

D. Preparation of extracts

Extracts were prepared in order to study their antioxidant activity. Ethanol, acetone and aqueous extracts of each of the leaves, stem bark and fruit were prepared by soaking the powders in the three solvents for 72h and the mixture was mixed with a sterile glass rod for every 24h., then the extracts were filtered using a Buckner funnel and Whatman No.1filter paper and concentrated by vacuum drying.

E. Pharmacological evaluation

1. Antioxidant activity

1.1 DPPH free radical scavenging activity

The DPPH radical scavenging activity of different solvent extracts of leaves, stem bark and fruit of *Holoptelea integrifolia* was assessed according to the previous method [7]. 1 ml of freshly prepared methanol solution of DPPH of 1 mM was mixed with different solvent extracts at different concentrations as 10–100 µg/ml. The reaction mixture was rattled and incubated at 37 °C for 30 min in dark place and absorbance was measured at 517 nm. In this activity, ascorbic acid considered as control and methanol as blank was also carried out simultaneously. The antioxidant BHT was used as a positive control in all assays. The inhibition of RSA was determined by the given equation.

$$\% \text{ RSA} = ([A_0 - A_S])/A_0 \times 100 \quad (1)$$

Where A_0 and A_S are the absorbance of the control (containing all reagents, except the test compound) and test compound respectively.

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1.2. Hydroxyl radical scavenging activity (HRSA)

HRS activity of different extracts of leaves, stem bark and fruits of *Holoptelea integrifolia* was evaluated as described previously [8] with small changes. 100 µl of different concentrations of extracts was dissolved in buffer as 10–100 µg/ml at 0.6 ml of PO_4 buffer at pH 7.4 along with 10 mM deoxyribose and 170 mM EDTA and the reaction was carried out by adding 100 µl of ascorbic acid (2 mM) and 100 µl of 10 mM H_2O_2. The reaction was stopped by the addition of 1 ml of 1% was heated at boiling water bath for 5 min after the incubation period of 20 min at 80–90 °C. The quantity of pink-colored chromogen was recorded at 532 nm and ascorbic acid as a control.

1.3. Nitric Oxide Radical Scavenging Activity

NOR Scavenging Activity of different extracts of leaves, stem bark and fruit for *Holoptelea integrifolia* was evaluated system [9]. Sodium nitroprusside (1ml of 10mM) were mixed with 1ml of HAEF of different concentration (50–250 µg/mL) in phosphate buffer (pH 7.4). The mixture was incubated at 25°C for 150 min. 1ml of Griess's reagent was added to 1 ml of the incubated solution. Absorbance was recorded at 546 nm. Ascorbic acid was utilized as the reference material. All tests were performed in triplicate and the results averaged. The % inhibition of OD was determined by the given formula.

% inhibition = Abs control - Abs sample/Abs control ×100% (2)

2. Antimicrobial activity

2.1 Agar-well diffusion assay

Clinical Pathogenic bacteria such as *Bacillus subtilis, Pseudomonas aeruginosa Micrococcus luteus , Eschericia coli* and *Proteus vulgaris* and fungal cultures (*Asperigillus niger, Aspergillus flavus, Tricoderma vibriae, Penicillium rubrum* and *Chaetomium globosum*) were procurred from MTCC, Chandigarh, India were used to perform the antimicrobial activity and antifungal by means of agar well-diffusion and MIC assay. All pure cultured bacteria were sub-cultured in the nutrient broth and incubated for 24 h at 30 °C in the rotatory shaker. The liquid bacterial culture was wipped on the Mueller–Hinton agar plates and 8 mm of hole was bored at the centre of plates [10]. The holes were filled with 100µl ethanolic solvent extracts of leaves, stem bark and fruit of *Holoptelea integrifolia* were tested in a concentration of 50 mg/100 µl and standard antibiotic (gentamycin) of 3 incubated at 37°C for bacterial strains about 24–48 h and standard antibiotic (Nystatin) for fungal strains about 3–5 days. The measurement of the inhibition zone diameter around the well were determined by the antibacterial and antifungal activities.

3. Anti - inflammatory Activity

3.1. Acute Anti inflammatory Activity

3.1.1 Formalin-induced Paw Oedema in mice

Acute inflammation was induced by injecting 0.1ml of 1% formalin (0.1 ml of 1% suspension in 0.9% saline) suspension in sub-plantar region and paw volume was measured 0,1,2,3,4 and 5 hours by using of Plethysmometer. All the treatment compounds were given 30 min, prior to formalin. Acute inflammation was induced in the right hind paw [11]. After injecting formalin, the first reading was taken at zero hr. and the protocol was repeated at first, second, third, four and fifth hours after formalin injection. The difference between 0 hr reading and one of the subsequent readings provide the actual oedema volume at the time [12]. The mean paw volume at different times was calculated and compared with the control and the percentage inhibition was determined with the help of the equation

Percent inhibition = $[(Vt - V_0)$ control $- (Vt - V_0)$ treated$]$ $/(Vt - V_0)$control $\times 100$ (3)

Group-I: Animlas injected distilled water and considered as control.

Group-II: Animals injected with concentration of 10 mg/kg of Diclofenac sodium i.p. and served as standard.

Group- III to VIII: Animals injected with concentration of 300 mg/kg of acetone, ethanol and water plants extracts.

3.2 Chronic Anti inflammatory Activity

3.2.1 Formalin Induced Paw Oedema

Albino wistar female rats weighing 170-250 mg/kg were Albino wistar female rats weighing 170-250 mg/kg were separated into eleven groups and each group contains six rats. Before the starting of the experiment, all the animals were fasted for 18 hrs and water was given ad libitum [18]. In animals of all the groups chronic inflammation was produced by sub plantar injection of 20µL of freshly prepared 2% suspension of formalin in normal saline in right hind paw of rat was used as the oedematogenic agent. Animals were treated with drugs for six consecutive days. The paw volume was measured using a plethysmometer before and 6 days after formalin tested in each group. The paw volume increase and percent of inhibition was calculated and compared with the control and the percentage inhibition was determined by using the equation

Percent inhibition = [(Vt–V_0) control – (Vt–V_0) treated] /(Vt–V0)control × 100 (4)

Group-I: Animlas injected distilled water and considered as control.

Group-II: Animals injected with concentration of 100 mg/kg of Diclofenac sodium i.p. and served as standard.

Group- III to VIII: Animals injected with concentration of 300 mg/kg of acetone, ethanol and water plants extracts.

4. Statistical analysis

All experiments were performed in triplicates and results were presented at the mean. Statistical analysis was conducted with multiple variance analysis (two-way ANOVA) with the Tukey's test by comparing every extracts with BHT for the Dose–response relationship.

III. RESULTS AND DISCUSSION

A. Preliminary phytochemical screening

Preliminary phytochemical screening of the leaves, stem bark and fruits of *Holoptelea integrifolia* investigations contains; unsaturated sterols or triterpenoides, Flavonoids, Carbohydrates or glycosides, Proteins and/ or amino acids, Tannins and Coumarin [14].

B. Antimicrobial activity

The results were obtained and compared with the scale developed by Arora and Bhardwaj [15]. The results of zone of inhibition and drug sensitivity were described as below.

Table I: Zone of Inhibition and drug sensitivity relationship

S. No.	Zone of Inhibition (m.m.)	Drug Sensitivity
1.	N.I. (below 6)	Insensitive
2 .	6 < 9	Less sensitive
3.	9 < 12	Moderate sensitive
4.	> 12	Highly sensitive

The antibacterial and antifungal activities of leaves, stem and fruit of *Holoptelea integrifolia* ethanolic extracts were determined by well-diffusion method. All the investigated *Holoptelea integrifolia* extracts showed antibacterial and antifungal activities (Tables I and II).

The results of leaves extract of *Holoptelea integrifolia* revealed significant antibacterial activity against *Bacillus subtilis* (7.00± 0.25mm), *Pseudomonas aeruginosa* (8.25± 0.25 mm) Micrococcus luteus (10.2± 0.10 mm), *Escherichia coli* (8.1± 0.25) and *Proteus vulgaris* (06± 0.25) (Table 2). The stem extract of *Holoptelea integrifolia* revealed significant antibacterial activity against *Bacillus subtilis* (11.2± 0.25 mm), *Pseudomonas aeruginosa* (12.25± 0.25 mm) *Micrococcus luteus* (13.25± 0.25 mm), *Escherichia coli* (12± 0.25) and *Proteus vulgaris* (10± 0.25). The antibacterial activity of leaves extract of *Holoptelea integrifolia* was obtained against *Bacillus subtilis* (9.1± 0.25 mm), *Pseudomonas aeruginosa* (9.25± 0.25mm) Micrococcus luteus (16 ± 0.25mm), *Escherichia coli* (09± 0.25) and Proteus vulgaris (08± 0.25). The highest antibacterial activity obtained by *Holoptelea integrifolia* was 12.25± 0.25 (stem extract) against *Pseudomonas aeruginosa*, 11.2± 0.25 (stem extract) against *Bacillus subtilis*, 16 ± 0.25 (fruit extract) against *Micrococcus luteus*, 12± 0.25 (stem extract) against *Escherichia coli* and 10± 0.25 (stem extract) against *Proteus vulgaris* (Table II).

Based on antifungal activity of the leaf, stem and fruit extracts of *Holoptelea integrifolia* reveals that the highest activity; 12± 0.25mm, 13.1± 2.25 mm, 8.5± 0.25 (stem extract) was obtained by *Holoptelea integrifolia* against *Asperigillus niger*, *Tricoderma vibriae and Chaetomium globosum* species respectively (Table II).

Table- II: Zone of inhibition of ethanolic extract of leaves, stem and fruit of *Holoptelea integrifolia*

S.No	Bacteria	Leaves extract	Stem extract	Fruit Extract	Standard Antibiotic
1.	*Pseudomonas aeruginosa* MTCC1688	8.25± 0.25	12.25± 0.25	9.25± 0.25	11.1± 0.25
2.	*Bacillus subtilus* MTCC 121	7.00± 0.25	11.2± 0.25	9.1± 0.25	13.4± 2.25
3.	*Micrococcus luteus* MTCC 1541	10.2± 0.10	13.25± 0.25	16 ± 0.25	20.1± 0.25
4.	*Eschericia coli* MTCC1687	8.1± 0.25	12± 0.25	09± 0.25	17.2± 1.25
5.	*Proteus vulgaris* MTCC 1771	06± 0.25	10± 0.25	08± 0.25	12.1± 2.25
	Fungi				
1.	*Asperigillus niger* MTCC 1344	07± 0.25	12± 0.25	10± 0.25	13.4± 1.25
2.	*Asperigillus flavus* MTCC 1444	ND	ND	ND	14.1± 2.25
3.	*Tricoderma vibriae* MTCC 1564	ND	13.1± 2.25	12± 0.25	16.1± 0.25
4.	*Pencillium rubrum* MTCC 214	ND	ND	ND	16.2± 1.25
5.	*Chaetomium globosum* MTCC 344	2.2± 0.25	8.5± 0.25	7.0± 0.25	12.1± 0.25

ND, not determined. These are the mean of three determinations.

C. Antioxidant activity

3.2.1. Antioxidant activity (DPPH free radical scavenging)

In several diseases like AIDS, Neurodegenerative diseases and cancer, the free radicals are responsible for causing those diseases and scavenging activity of antioxidants is responsible for the control of those diseases. The most commonly and sensitive method used for screening of antioxidants was DPPH assay and it determine the antioxidant activity of different plant, algal and fungal extracts [16].

Table III: DPPH free radical scavenging of different extracts of leaves and bark of *H.integrifolia*

Test Compounds	% Inhibition for Absorbance (Mean ± SEM)				
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
Ascorbic acid	1.102 ± 0.008 (34.71%)	0.517 ± 0.002 (69.54%)	0.199±0.002 -84.46%	0.137 ± 0.002 (91.89%)	0.116 ± 0.001 (93.14%)
HI -LV-ETH	1.320 ± 0.005 (27.85%)	0.835 ± 0.002 (50.62%)	0.551 ± 0.004 (67.41%)	0.221 ± 0.002 (86.93%)	0.155 ± 0.002 (90.83%)
HI -LV-ACET	1.243 ± 0.008 (27.08%)	0.972 ± 0.005 (42.63%)	0.645 ± 0.002 (61.85%)	0.288 ± 0.004 (82.96%)	0.191 ± 0.001 -88.70%
HI -LV-WATE	1.300 ± 0.005 (23.12%)	0.968 ± 0.004 -42.81%	0.712 ± 0.004 (57.95%)	0.438 ± 0.001 (74.09%)	0.207 ± 0.001 -87.69%
HI- BRK-ETH	1.328 ± 0.001 -21.46%	1.117 ± 0.044 (33.94%)	0.788 ± 0.004 (53.40%)	0.558 ± 0.003 (67.12%)	0.301 ± 0.006 (82.19%)
HI -BRK-ACET	1.275 ± 0.002 (24.60%)	1.073 ± 0.030 (36.60%)	0.915 ± 0.002 (45.89%)	0.685 ± 0.002 (59.49%)	0.515 ± 0.002 (69.54%)
HI -BRK- WATE	1.255 ± 0.002 (25.78%)	0.865 ± 0.002 (48.84%)	0.458 ±0.001 (72.91%)	0.209 ±0.001 (87.69%)	0.149 ± 0.002 (91.42%)

HI :*Holoptelia integrifolia* , LV :Leaves, ETH: ethanol extract, ACE: acetone, WATE: water extract, BRK: bark

The ethanol, Acetone and water extract of leaf and Bark of *Holoptelia integrifolia* showed DPPH radical scavenging activity depends on the concentration manner (Table III). The DPPH scavenging activity was found to be 90.83%, 88.70%, 87.69% % and 93.14% at 250 µg/ml for ethanol, acetone, water extract and Vitamin C for leaves of *Holoptelia integrifolia* (Table III). The DPPH scavenging activity was found to be 82.19%%, 69.54%, 91.42 % and 93.14 % at 250 µg/ml for ethanol, acetone and water extract and Vitamin C for bark of *Holoptelia integrifolia*. The maximum scavenging activity (90.83% and 91.42%) was provided by ethanol extract of leaves and water extract of bark for *Holoptelia integrifolia*.

1. Antioxidant activity (Hydroxyl Radical Scavenging)

The ethanol, Acetone and water extract of leaf and Bark of *Holoptelia integrifolia* showed Hydroxyl Radical Scavenging in a concentration– dependent manner (Table IV). The Hydroxyl Radical Scavenging was found to be 74.43%, 65.66%, 70.42% and 80.20% at 250 µg/ml for ethanol, acetone and water extract and Vitamin C for leaves of *Holoptelia integrifolia* (Table IV). The DPPH scavenging activity was found to be 66.16%, 69.42%, 72.68% and 80.20% at 250 µg/ml for ethanol, acetone and water extract and Vitamin C for bark of *Holoptelia integrifolia*. The maximum scavenging activity (74.43% and 72.68%) was provided by ethanolic extract of leaves and water extract of bark for *Holoptelia integrifolia.*

Table IV: Hydroxyl Radical scavenging of different extracts of leaves and bark of *Holoptelea integrifoli*

Test Compounds	% Inhibition for Concentration (Mean ± SEM)				
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
Ascorbic acid	0.209 ± 0.002 (49.37%)	0.149 ± 0.002 (62.65%)	0.113 ± 0.002 (71.67%)	0.094 ± 0.001 (76.44%)	0.078 ± 0.001 (80.20%)

HI -LF-ETH	0.274 ± 0.001 (31.57%)	0.215 ± 0.002 (46.11%)	0.176 ± 0.002 (55.88%)	0.122 ± 0.003 (69.42%)	0.102 ± 0.002 (74.43%)
HI -LF-ACET	0.294 ± 0.002 (27.06%)	0.233 ± 0.002 (41.60%)	0.186± 0.001 (54.13%)	0.153 ± 0.002 (61.65%)	0.137 ± 0.001 (65.66%)
HI -LF-WATE	0.266 ± 0.004 (34.08%)	0.218 ± 0.002 (45.11%)	0.172 ± 0.001 (56.89%)	0.134 ± 0.002 (66.41%)	0.117 ± 0.002 (70.42%)
HI- BRK-ETH	0.256 ± 0.004 (35.83%)	0.201 ± 0.003 (49.62%)	0.179 ± 0.002 (56.14%)	0.158 ± 0.002 (60.40%)	0.133 ± 0.001 (66.16%)
HI -BRK-ACET	0.271 ± 0.001 (32.08%)	0.224 ± 0.001 (43.10%)	0.163 ± 0.002 (59.14%)	0.141 ± 0.001 (64.66%)	0.121 ± 0.002 (69.42%)
HI -BRK- WATE	0.248 ± 0.002 (37.84%)	0.193 ± 0.003 (50.62%)	0.162 ± 0.002 (59.14%)	0.130 ± 0.001 (67.41%)	0.108 ± 0.002 (72.68%)

HI- Holoptelia integrifolia , LV- Leaves , ETH- ethanol extract, Ace- acetone, WATE- water extract, BRK- bark

2. Antioxidant activity (Nitric Oxide Radical Scavenging)

The ethanol, Acetone and water extract of leaf and Bark of *Holoptelia integrifolia* showed Nitric Oxide Radical Scavenging activity in a concentration– dependent manner (Table V). The Nitric Oxide Radical Scavenging was found to be 73.39 %, 67.57%, 70.76% and 80.11% at 250 µg/ml for ethanol, acetone and water extract and Vitamin C for leaves of *Holoptelia integrifolia* (Table V). The DPPH scavenging activity was found to be 64.91 %%, 70.76%, 72.80% and 80.11% at 250 µg/ml for ethanol, acetone and water extract and Vitamin C for bark of *Holoptelia integrifolia*. The maximum scavenging activity (73.39% and 72.80 %) was provided by ethanolic extract of leaves and water extract of bark for *Holoptelia integrifolia*.

Table V: Nitric Oxide scavenging of different extracts of leaves and Bark of *Holoptelea integrifolia*

Test Compounds	% Inhibition for Concentration (Mean ± SEM)				
	50 µg/ml	100 µg/ ml	150 µg/ ml	200 µg/ ml	250 µg/ ml
Ascorbic acid	0.158 ± 0.004 (53.80%)	0.135 ± 0.002 (60.52%)	0.115 ± 0.002 (66.37%)	0.100 ± 0.002 (70.76%)	0.068 ± 0.001 (80.11%)
HI -LF-ETH	0.195 ± 0.002 (42.98%)	0.160 ± 0.002 (53.21%)	0.136 ± 0.004 (60.23%)	0.115 ± 0.002 (66.37%)	0.091 ± 0.016 (73.39%)
HI -LF-ACET	0.193 ± 0.001 (43.56%)	0.168 ± 0.001 (50.87%)	0.150 ± 0.002 (56.14%)	0.135 ± 0.002 (60.52%)	0.111 ± 0.004 (67.57%)
HI -LF-WATE	0.181 ± 0.001 (40.35%)	0.158 ± 0.001 (53.80%)	0.138 ± 0.004 (59.64)	0.116 ± 0.003 (66.08%)	0.100 ± 0.002 (70.76%)
HI -BRK-ETH	0.196 ±0.004 (42.69%)	0.171 ± 0.001 (50.00%)	0.155 ± 0.002 (54.67%)	0.131 ± 0.001 (61.69%)	0.120 ± 0.002 (64.91%)
HI -BRK-ACET	0.181 ±0.001 (47.07%)	0.165 ±0.002 (51.75%)	0.141 ± 0.001 (58.77%)	0.120 ±0.002 (64.91%)	0.100 ± 0.002 (70.76%)
HI -BRK-WATE	0.181 ± 0.001 (40.35%)	0.158 ± 0.001 (53.80%)	0.138 ± 0.004 (59.64)	0.116 ± 0.003 (66.08%)	0.093 ± 0.003 (72.80%)

3. Acute Anti inflammatory Activity study:

Following the up and down method, the ethanol, acetone and water extracts of leaves and bark of *Holoptelia integrifolia* at a dose of 300 mg/kg were selected in the present study.

In Formalin-induced Inhibition of activity in mice, the inhibition percentage of the control, standard and test compounds are shown in Table V. The entire test compounds were compared with diclofenac as a standard at a concentration of 10 mg/kg for anti-inflammatory activity [17]. In this study, diclofenac showed 60.02% inhibition of inflammation at 3rd h when compared to control.

Ethanol, acetone and water extracts for leaves of *Holoptelia integrifolia* (300 mg/kg) showed significant inhibition of inflammation with 44.57%, 36.55% and 36.93%, respectively (Table V). Whereas, 300 mg/kg Ethanol, acetone and water extracts for bark of *Holoptelia integrifolia* showed 42.57%, 31.73 and 38.33% respectively inhibition of oedema, respectively at 3rd h when compared with control [18]. The results of test compounds were found to be statistically significant at value *** P<0.001, ** P<0.01, * P<0.05.

Table V: Effect of *H. integrifolia* plant extracts on formaline induced writhing in mice

Groups	Treatment	Mean no of writhing ±SEM	% Inhibition of writhes
Group-I	Saline	40.50 ± 1.25	-
Group-II	Diclofenac (10 mg/Kg)	15.59 ± 0.92***	60.02%
Group-III	Leaf-Ethanol (300 mg/Kg)	23.00 ± 1.06***	44.57%
Group-IV	Leaf--Acetone (300 mg/Kg)	25.33 ± 1.38***	36.55%
Group-V	Leaf--Water (300 mg/Kg)	26.17 ± 1.49***	36.93%
Group-VI	Bark- Ethanol (300 mg/Kg)	24.83 ± 1.30***	42.57%
Group-VII	Bark-Acetone (300 mg/Kg)	28.33 ± 1.66***	31.73%
Group-VIII	Bark- Water (300 mg/Kg)	23.59 ± 1.43***	38.33%

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

4. Chronic Anti inflammatory Activity study

Following the up and down method, the ethanol, acetone and water extracts of leaves and bark of *Holoptelia integrifolia* at a concentration of 300 mg/kg were selected in the present study.

In Formalin-induced Paw Oedema activity in Albino wistar Rats, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in Table V. The entire test compounds were compared with diclofenac as a standard at a concentration of 100 mg/kg for antiinflammatory activity [19]. In this study, diclofenac showed 82.40% inhibition of inflammation at 5th h when compared to control.

Ethanol, acetone and water extracts for leaves of *Holoptelia integrifolia* (300 mg/Kg) showed significant inhibition of inflammation with 65.66%, 20.60% and 54.07%, respectively (Table VI). Whereas, 300 mg/Kg Ethanol, acetone and water extracts for bark of *Holoptelia integrifolia* showed 59.22%, 28.75 and 20.60 % respectively inhibition of oedema, respectively at 5th h when compared with control [20]. The results of test compounds were found to be statistically significant at value P<0.05.

Table VI: Effect of *H. integrifolia* plant extracts on formalin-induced paw oedema (chronic) in rats

Groups	Treatment	Initial Paw Volume	Paw Volume after Six Days	Increase in Paw Volume	% of Inhibition
Group-I	Saline	1.28 ± 0.07	3.61 ± 0.12	2.33 ± 0.06	-
Group-II	Diclofenac (10 mg/Kg)	1.23 ± 0.04	1.65 ± 0.05	0.41 ± 0.07	82.40%
Group-III	Leaf-Ethanol (300 mg/Kg)	1.26 ± 0.03	2.00 ± 0.06	0.80 ± 0.12	65.66%
Group-IV	Leaf--Acetone (300 mg/Kg)	1.21 ± 0.06	3.21 ± 0.24	1.85 ± 0.16	20.60%
Group-V	Leaf--Water (300 mg/Kg)	1.25 ± 0.06	2.25 ± 0.16	1.07 ± 0.14	54.07%
Group-VI	Bark- Ethanol (300 mg/Kg)	1.31 ± 0.08	2.26 ± 0.10	0.95 ± 0.14	59.22%
Group-VII	Bark-Acetone (300 mg/Kg)	1.23 ± 0.06	2.90 ± 0.14	1.66 ± 0.17	28.75%
Group-VIII	Bark- Water (300 mg/Kg)	1.26 ± 0.06	3.11 ± 0.08	1.85 ± 0.11	20.60%

Results are expressed on mean + SEM (n=6) from four observations of paw volume was measured after six days.

IV. CONCLUSION

In the present work, the different extracts of leaves, bark and fruits of *Holoptelia integrifolia* was assessed for its antimicrobial, antioxidant and anti-inflammatory activity. The different extracts of *Holoptelea integrifolia* leaves, stem bark and fruits were determined as a potent natural source of antimicrobial, antioxidant and wound healing potential. The results of Antimicrobial activity of ethanol extract of leaves, bark and fruits for *Holoptelia integrifolia* shown excellent antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Eschericia coli* and *Proteus vulgaris* but the antifungal activity shown against *Asperigillus niger, Tricoderma vibriae and Chaetomium globosum*. The results of Antioxidant activity of ethanol, acetone and water of leaves and bark for *Holoptelia integrifolia* have shown anti-oxidant activity at the at 250 µg/ml concentration. Thus the present study concludes that the different extracts of leaves and bark of *Holoptelia integrifolia* have anti-inflammatory activity in acute and chronic phase of inflammation in laboratory animals at the dose of 300 mg/kg without ulcerogenic effect. Further investigation is needed to know the protective effect of leaves and bark of *Holoptelia integrifolia* using different animal models.

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CONFLICT OF INTEREST

The authors confirmed that they do not have any conflict of interest.

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