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Research Article

Evaluation of *In-vitro* Anti-inflammatory and Antibacterial Potential of Methanolic Leaf Extracts of *Elytranthe parasitica* (L.) Danser (Loranthaceae): A Hemiparasitic Angiosperm

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ABSTRACT

Elytranthe parasitica (L.) Danser is a hemiparasitic angiosperm shrub commonly called *Macrosolen parasiticus* and a member of the Loranthaceae family. The members of Loranthaceae are utilized globally in conventional medicine to cure a variety of ailments. The current study aimed to assess the qualitative screening of *E. parasitica* for its secondary metabolites and also *in-vitro* evaluation of anti-inflammatory and antibacterial potential. The fresh leaf samples were collected and extracted with the help of a soxhlet extractor. The *in-vitro* anti-inflammatory potential was determined by protein denaturation and HRBC membrane stabilization assays, and evaluation of antibacterial properties by agar well diffusion assay. The results of the qualitative evaluation of phytoconstituents revealed a presence of wide range of secondary metabolites. The methanolic leaf extracts of *Elytranthe parasitica* exhibited significant protein denaturation activity with 82.46 ± 1.37 % of inhibition of protein denaturation at 250 μg mL^{-1} concentration and showed significant membrane stabilization activity with 65.57 ± 2.60 % of membrane stabilization at 500 μg mL^{-1} concentration. It also shows considerable antibacterial potential with the maximum inhibition zone against *Xanthomonas campestris* (18.16 ± 0.44 mm) followed by *Escherichia coli* (15.33 ± 0.88 mm), *Staphylococcus aureus* (11.16 ± 0.44 mm), and *Salmonella typhi* (10.66 ± 0.66 mm) at higher concentrations. To conclude, it was found that *E. parasitica* (L.) Danser has a variety of secondary metabolites that have potent anti-inflammatory and antibacterial properties based on the experiments carried out.

INTRODUCTION

Loranthaceae is the largest mistletoe family, with 73 genera and more than 900 species.[1] The members of Loranthaceae are utilized globally in conventional medicine to cure a variety of ailments, including diabetes, condyloma, haemorrhoids, arthritis, inflammatory diseases, as well as breathing, neurological difficulties, and several cancers.[2] Phytochemical investigations of several Loranthaceae members have shown various types of phytoconstituents, such as alkaloids, gallic acid, arginine, histamines, tannins,polysaccharides, terpenoids,steroids, polypeptides, lectins, flavonoids, and glycosides,[3] and recently identified, a novel polyhydroxylated flavanocoumarin derivative, known as loranthin from *Plicosepalus acacia*[4] and a novel diarylpropanoid discovered as macrotricolorin along with three diarylheptanoids including bisdemethoxy curcumin, demethoxy curcumin, and curcumin were isolated from the *Macrosolen tricolor*.[5]

Inflammation is a body responds to damage, infections, or destructions characterized by heat, redness, discomfort, swelling, and altered physiological processes. Inflammation is an ordinary defensive reaction to tissue damage brought on by physical trauma, toxic chemicals, or pathogenic microorganisms.[6] The body reacts in order to neutralize or eliminate the invaders, get rid of the irritations, and prepare the environment for tissue restoration. Chemical mediators released from injured tissue and migrating cells serve as its catalyst. Nonsteroidal anti-inflammatory medicines, which are

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frequently a treatment for inflammatory disorders, have several adverse effects, particularly stomach irritation that can result in gastric ulcers.[7] Due to the negative side effects of pharmaceutical medications, using complementary natural treatments is an alternate course of action. However, there has been little research on the use of medicinal herbs in treating inflammation.

Around the world, the prevalence of infectious diseases is steadily rising and they are a leading cause of death. Treatments using antimicrobial medications are made more challenging by the overuse of antibiotics, the prevalence of adverse effects, the challenge of developing novel agents, and particularly the growing issue of resistance. A global threat to the welfare of people, animals, and the environment has emerged as a result of the recent acceleration of the antibiotic resistance problem caused by the excessive and incorrect use of antibiotics. Antibiotic resistance is thought to be a contributing factor in 700,000 deaths annually across the globe. The pace of therapy for infectious diseases is not kept up with the antibiotics that are released into the market, on the other hand. Investigation of new antimicrobial drugs is necessary due to the rapid emergence of bacteria resistant to antibiotics produced and the rise in infectious disease-related mortality.[8]

Elytranthe parasitica (L.) Danser is a hemiparasitic angiosperm shrub commonly called *Macrosolen parasiticus* and it is a member of the family Loranthaceae (the largest family of mistletoes). It grows abundantly in the Western Ghats regions of Karnataka, especially on jackfruit, mango, neem, and peepal trees.[9] Earlier studies on *E. parasitica* concentrated on their anticancer properties against MCF-7 (Human breast cancer cell line),[10] EAC (Ehrlich ascites carcinoma cells) *in-vivo* models,[11] HepG2 (Hepatocellular carcinoma),[12] HCT 116 (Colorectal cancer cells),[13] and PC-3 (Prostate cancer cells),[14] and antioxidant activities.[15-16] However, the anti-inflammatory and antibacterial properties of *E. parasitica* have not been studied scientifically. This study aims to explore the *in-vitro* anti-inflammatory and antibacterial potential of the methanolic leaf extract of *E. parasitica*.

Material and Methods

Sample Collection

Leaf samples of *E. parasitica* growing on the *Terminalia paniculata* was collected from near Kavaledurga fort, Shivamogga district, and taxonomically identified by Dr. Ravikumar taxonomist and HOD Department of Botany, AVK College Hassan, and a Herbarium of a plant sample is prepared and deposited with number KU/AB/RN/KPS-1.

Preparation of Extracts and Screening of Phytoconstituents

The collected leaf samples were carefully cleaned under running water and shade dried for about 22 to 25 days and mechanically pulverized. The powdered leaf samples were subjected to soxhlet extraction with petroleum ether (PE), chloroform (CH), and methanol (ME). Obtained crude extracts were air-dried and kept in vials at 4°C temperature. Preliminary screening of phytochemicals using the standard method.[17]

In-vitro Screening of Anti-inflammatory Potential

Inhibition of Protein Denaturation Assay

In 0.5 mL test solution is made up of 0.45 mL of BSA (5% w/v aqueous phase) and 0.05 mL of test solution (250 µg/mL). BSA (5% w/v aqueous phase) and distilled water are combined to make up the test control solution, which has a volume of 0.5 mL.

In 0.45 mL of distilled water and 0.05 mL of the sample solution (250 µg mL^{-1}) make up the final control (0.5 mL). In 0.45 mL of BSA (5%w/v aqueous solution) and 0.05 mLof aspirin (250 µg mL^{-1}) make up the standard solution (0.5 mL).

Using 1N hydrochloric acid, the pH of each of the aforementioned solutions was brought to 6.3. The samples were kept in an incubator at 37°C. for about 20 minutes and the samples were kept at 57°C for 3 minutes by raising the temperature. After cooling, 2.5 mL of phosphate buffer saline was included in the aforementioned solutions. The absorbance was recorded spectrophotometrically at 416 nm.

$$\%\text{protein denaturation activity} = [(Ac - At)/Ac] \times 100$$

where, Ac and At is the absorbance of the control and sample, respectively

HRBC Membrane Stabilization Assay

The premise behind this technique is the stability of the human red blood cell membrane by membrane lysis brought on by hypotonicity. Blood samples (2 mL) were taken from healthy participants and combined with an equivalent amount of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water). The mixture was then centrifuged at 3000 rpm. A 10% v/v suspension of normal saline was made and stored at 4°C undisturbed earlier being used to wash the packed cells with an isosaline solution. The control was distilled water instead of hyposaline to induce 100% hemolysis. Different quantities of *E. parasitica* extract were combined separately with 1-mL of phosphate buffer, 2 mL of hyposaline, and asperin as standard (100, 200, 300, 400, and 500 µg/0.5 mL). After centrifuging all of the assay mixtures at 3000 rpm for 20 and 30 minutes at 37° C, the supernatant solution's haemoglobin content was determined by a spectrophotometer at 560 nm. Using the following formula, the % of stabilization was determined.

$$\%\text{ of stabilization } [(Ac - As)/Ac] \times 100$$

Where A_c = Absorbance of control and A_s = Absorbance of the test sample

Antibacterial Assay

Antibacterial analysis of methanolic leaf extracts against some selected plant and human pathogenic bacterial strains was performed by using agar well diffusion assay according to Balouiri M *et al.*,[18] along with slight modifications. After 24 hours of old cultured bacterial strains on a nutrient broth were swabbed evenly on solidified nutrient agar (media) plates by using sterile cotton swabs. Then, 6 mm diameter wells were punched by a sterilized cork borer. Each well of the plate was loaded with 20 µL of methanolic leaf extracts at different concentrations viz., 10 mg of each crude extract was dissolved in 1-mL of DMSO and made into concentrations of 100, 50, and 25%. Standard drug ciprofloxacin, 1 mg/mL, utilized as a + ve control, and DMSO, utilized as a –ve control, were added separately into the respective labeled wells of the plates. The test bacterial strains consist of one gram positive pathogenic bacterial strain *Staphylococcus aureus* (MTCC-4734)and three gram negative pathogenic bacteria *Escherichia coli* (MTCC-1599), *Xanthomonas campestris* (MTCC-228), and *Salmonella typhi* (MTCC- 734). The plates were inoculated and placed in an incubator at 35 to 37°C for about 24 hours to determine the inhibition zone. The experiments were triplicated to get average values.

Results

Qualitative Screening of Secondary Metabolites

The results of screening different extracts of *E. parasitica* showed a variety of secondary metabolites including phenols, triterpenoids, alkaloids, flavonoids, glycosides, flavonoids, steroids, and saponins (Table 1).

Table 1: Qualitative screening of secondary metabolites of *E. parasitica*

Name of the secondary metabolites	*Petroleum ether*	*Chloroform*	*Methanol*
Alkaloids	-	-	+
Flavonoids	-	-	+
Glycosides	+	+	+
Phenols	-	-	+
Triterpenoids	-	+	+
Steroids	+	+	+
Saponins	-	-	+

In-vitro Anti-inflammatory Property

Inhibition of Protein Denaturation Assay

In-vitro anti-inflammatory activity of methanolic leaf extracts of *E. parasitica* was evaluated by using the inhibition of protein denaturation assay at various concentrations (50, 100, 150, 200, and 250 µg/mL) was shown in Table 2 and Fig. 1. The results of the inhibition of the protein denaturation assay exhibited dose-dependent activity. The methanolic leaf extracts of *E. parasitica* exhibited significant inhibition of protein denaturation activity with 82.46 ± 1.37 % of inhibition of protein denaturation at 250 µg mL^{-1} and its effects were contrasted with those of the common anti-inflammatory medication, aspirin exhibited the maximum inhibitory activity with 94.97 ± 0.752 % of inhibition of protein denaturation at the same concentration.

Table 2: Effect of leaf methanolic extract of *E. parasitica* on inhibition of protein denaturation

Sl. No	*Concentration (µg/mL)*	*E. parasitica extract denaturation %*	*Standard aspirin denaturation%*
1	50	20.47 ± 3.35	43.20 ± 2.13
2	100	34.81 ± 3.18	53.60 ± 0.92
3	150	50.56 ± 1.63	67.51 ± 1.12
4	200	66.44 ± 1.17	81.68 ± 1.20
5	250	80.94 ± 1.71	95.39 ± 0.60

Note: Values are expressed in Mean ± Standard Error of Mean
The experiments were triplicated (n=3)

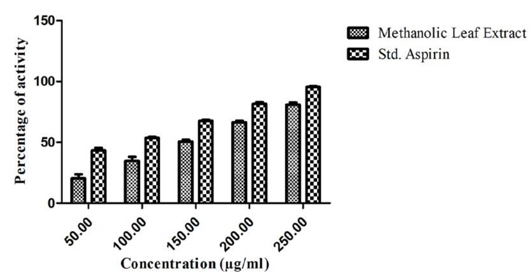

Fig. 1: Inhibition of the protein denaturation activity of leaf methanolic extract of *E.parasitica*

HRBC Membrane Stabilization Technique

In this investigation, the efficacy of *E. parasitica* methanolic leaf extracts to prevent RBCs from hemolyzing due to heat was used to measure their effectiveness in stabilizing membranes. The extracts' activity was concentration-dependent. The methanolic leaf extracts of *E. parasitica* exhibited significant membrane stabilization activity with 65.57 ± 2.60 % of membrane stabilization at 500 µg mL^{-1} concentration and standard medication aspirin exhibited the maximum membrane stabilization activity with 74.66 ± 0.83 % of membrane stabilization at the same concentration (Table 3 and Fig. 2). These outcomes might be attributed to the presence of different phytoconstituents.

Antibacterial Activity

The antibacterial activity of leaf methanolic extract of *E. parasitica* against selected bacterial pathogenic bacterial strains was shown in Table 4, Figs 3, and 4. Leaf methanolic extract of *E. parasitica* exhibited considerable and dose-dependent antibacterial activity and it shows a maximum inhibition zone against *Xanthomonas campestris*

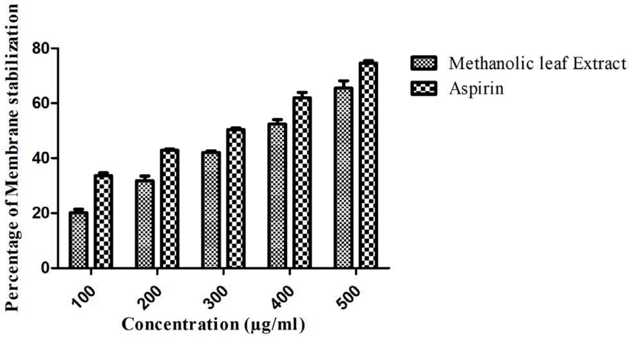

Fig. 2: HRBC membrane stabilization activity of leaf methanolic extract of *E. parasitica*

Table 3: Effect of leaf methanolic extract of *E. parasitica* on membrane stabilization activity

Sl. No	*Concentration (µg/mL)*	*E. parasitica extract membrane stabilization%*	*Standard aspirin membrane stabilization%*
1	100	20.18 ± 1.17	33.65 ± 0.98
2	200	31.77 ± 1.71	42.91 ± 0.38
3	300	42.07 ± 0.56	50.34 ± 0.59
4	400	52.51 ± 1.60	61.98 ± 1.95
5	500	65.57 ± 2.60	74.66 ± 0.83

Note: Values are expressed in Mean ± Standard Error of Mean
The experiments were triplicated (n=3)

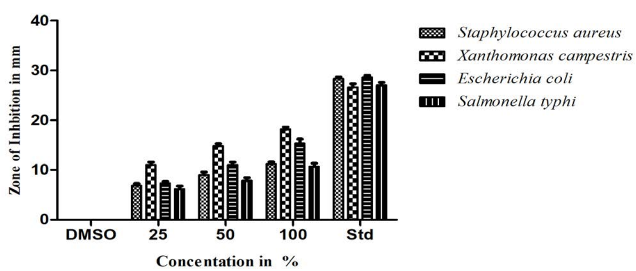

Fig. 3: Antibacterial potential of the leaf methanolic extract of *E. parasitica* against pathogenic bacterial strains.

(18.16 ± 0.44 mm) followed by *Escherichia coli* (15.33 ± 0.88 mm), *Staphylococcus aureus* (11.16 ± 0.44 mm), and *S. typhi* (10.66 ± 0.66 mm) at higher concentrations. The activity of leaf extracts against bacterial strains is lesser when compared with the common antibiotic medication ciprofloxacin and DMSO does not show any activity against all selected bacterial strains.

Discussions

Preliminary phytochemical evaluation of different solvent extracts of *E. parasitica* exhibited the existence of various secondary metabolites (Table 1). These secondary metabolites have numerous pharmacological properties such as antibacterial, anti-inflammatory, hepatoprotective, anti-allergic, anticancer, anti-diabetic, insecticidal, anti-malarial, and anti-viral properties.[19]

The use of steroidal and nonsteroidal anti-inflammatory medicines in the usual treatment of inflammatory illnesses results in severe adverse effects. Since natural anti-inflammatory compounds are typically thought to be safer and more tolerated than conventional anti-inflammatory medications, efforts have been made in recent years to find new anti-inflammatory molecules from natural sources[20] and also the use of animals in for clinical and pharmacology studies has some drawbacks, including ethical issues with its use and a lack of justification, when more suitable alternatives are accessible or may be examined.[7] So, in this current work, the anti-inflammatory property of the methanolic leaf extract of *E. parasitica* was determined *in-vitro*, using the protein denaturation and HRBC membrane stabilization bioassay.

Methanolic leaf extracts of *E. parasitica* exhibited a maximum protein denaturation inhibitory activity. It has been reported that major compounds in GCMS activity such as dihydrochrysin; n-hexadecanoic acid; 9-hexadecenoic acid, and 9,12-octadecadienoic acid have anti-inflammatory properties.[14] One of the factors that contribute to inflammation is protein denaturation. Protein *in-vivo* denaturation may be the cause of the formation of autoantigens in inflammatory diseases. Denaturation may occur as a result of modifications in electrostatic, hydrogen, hydrophobic, and disulfide bonding.[21] According to the findings, the methanolic extract of *E. parasitica* was able to regulate the formation of autoantigen, which prevents protein denaturation. Its impact was contrasted to that of the common medication Aspirin.

Methanolic leaf extracts of *E. parasitica* showed a membrane stability effect by preventing the lysis of the erythrocyte membrane caused by hypotonicity, an analogy between the liposomal membrane and the erythrocyte membrane exists.[22] Its stabilization suggests that the extract might also be able to maintain lysosomal membrane stability. Lysosome membrane stability is essential for controlling the inflammatory response because it prevents the release of lysosomal elements of active neutrophils, such as bactericidal enzymes and proteases, which when released extracellularly, cause more tissue inflammation and damage.[23] Though the specific mechanism of the extract's membrane stability is still unknown, cell shrinkage caused by the osmotic losses of intracellular electrolyte and fluid constituents or interactions with membrane proteins may cause hypotonicity-induced hemolysis.[24,25] The efflux of these intracellular constituents may be stimulated or enhanced by the processes that the extract may suppress. According to the aforementioned findings, *E. parasitica* methanolic extracts exhibit anti-inflammatory properties.

The leaf methanol extract of *E. parasitica* showed

Table 3: Antibacterial potential of the leaf methanolic extract of *E. parasitica* against pathogenic bacterial strains.

S. No.	*Name of the bacterial strains*	*Inhibition zone in mm*				
		Concentration in percentage			*Standard (Ciprofloxacin)*	*Control (DMSO)*
		100%	*50%*	*25%*		
1	*Staphylococcus aureus*	11.16 ± 0.44	9.00 ± 0.57	06.83 ± 0.44	28.33 ± 0.33	00
2	*Xanthomonas campestris*	18.16 ± 0.44	14.83 ± 0.44	11.00 ± 0.57	26.66 ± 0.66	00
3	*Escherichia coli*	15.33 ± 0.88	11.00 ± 0.57	07.33 ± 0.33	28.66 ± 0.33	00
4	*Salmonella typh*i	10.66 ± 0.66	07.83 ± 0.60	06.16 ± 0.60	27.00 ± 0.57	00

Note: Values are expressed in Mean ± Standard Error of Mean
The experiments were triplicated (n=3)

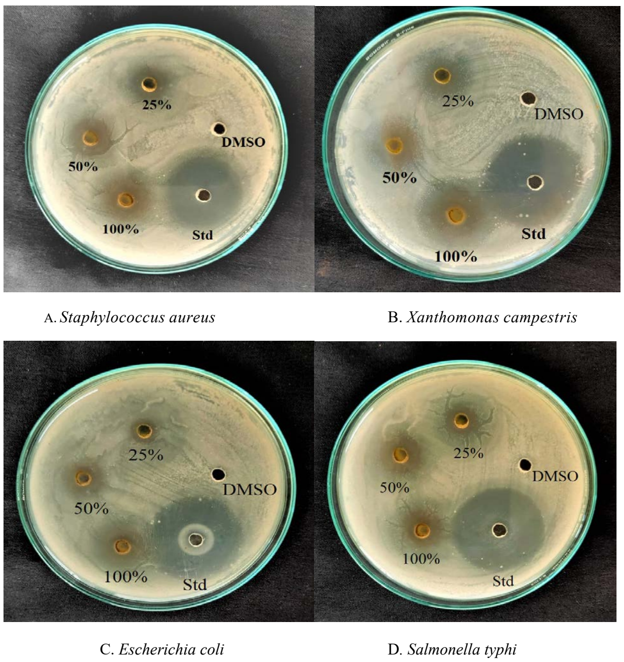

Fig. 4: Antibacterial activity of *E. parasitica* leaf methanolic extract of against selected pathogenic bacterial strains.

considerable antibacterial activity against selected pathogenic bacterial strains. Whereas the major reported compounds in GCMS activity such as agaricic acid; glyceryl 1,2-dipalmitate; 11-octadecenoic acid, methyl ester; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; dihydrochrysin and octadeca-9,12-dienoic acid are previously reported to have antibacterial activity.[14] Similarly, there is several Loranthaceae members are previously reported to have antibacterial properties.[26-32] The broad-spectrum antibacterial properties found in this investigation, which are consistent with the findings of other studies, were caused by the occurrence of biologically active phytocompounds such as tannins, steroids, terpenoids, phenols, flavonols, alkaloids, and others (either separately or collectively) in the examined plant extracts.[33, 34] Some phytoconstituent's antibacterial mechanisms could be summarised as follows: Tannins may function by deactivating microbial adhesins, enzymes, and cellular membrane transport proteins.[35] By changing the bacterial cell membranes, flavonoids prevent energy consumption and the production of nucleic acids,[36] alkaloids, terpenoids, and phenols via rupturing microbial cell membranes and/or suppressing deoxyribonucleic acid (DNA) formation.[37]

To conclude, it was found that *E. parasitica* (L.) Danser has a variety of secondary metabolites that have potent anti-inflammatory and antibacterial properties based on the experiments carried out. This provides scientific support for further research into the plant's lead compounds and efforts to conduct *in-vivo* models.

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