

Full Length Research Paper

Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc.

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Ethanol extract of leaf, stem and flower of *Wedelia trilobata* was assessed for its antimicrobial, antioxidant and anti-inflammatory activity and phytochemical screening. Total phenolic content was assessed using Folin-Ciocalteu's method. The antioxidant activity was determined by measuring the scavenging activity of DPPH radical and FRAP assay. The antimicrobial efficacy was determined using paper disc method against different fungi and bacteria. Sensitivity in terms of zones of inhibition and phytochemical composition of the all parts extracts were also determined. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation, membrane stabilization assay and proteinase inhibitory assay. Aspirin was used as a standard drug for the study of anti-inflammatory activity. The results show that, all parts extracts effective against all the bacteria tested, whereas all the extracts were failure in inhibiting the growth of all *Alternaria* sp., *Cercospora carthami* and *Nigrospora oryzae*, but other fungi also were showed moderate inhibition against all the three extracts. Phytochemical analysis revealed the presence of tannins, flavonoids, terpenoids, phenols and saponins. Leaf and stem ethanol fractions showed highest total Phenolic content. The leaf and stem ethanol extract possessed strong scavenging activity in both DPPH and FRAP methods. In DPPH and FRAP method, the leaf and stem had showed free radical inhibition of 86, 82 and 630.72, 508.81 respectively. The leaf and stem ethanol extract also showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization with 87.14 and 86.76 and 78.11, 74.17 g/ml respectively. Proteinase activity was also significantly inhibited by the leaf (84.19 g/ml) and stem (81.84 g/ml). From the result, it is concluded that phytochemicals (tannins, flavonoids, terpenoids, phenols and saponins) present in the *W. trilobata* extract may be responsible for the antimicrobial, antioxidant and anti-inflammatory activity.

Key words: *Wedelia trilobata*, antimicrobial, antioxidant, anti-inflammatory, phytochemicals.

INTRODUCTION

The increase in prevalence of multiple drug resistance has shown the development of new synthetic antimicrobial, antioxidative and anti-inflammatory drugs; moreover, the new drug is necessary to search for new antimicrobial, antioxidant and anti-inflammatory sources from alternative sources. Phytochemicals from medicinal

plants showing antimicrobial, antioxidant and anti-inflammatory activities have the potential of filling this need because their structures are different from those of the more studied plants, while those with more action may likely differ (Fabricant and Fanworth, 2001). In this growing interest, many of the phytochemical bioactive compounds from a medicinal plants have shown many pharmacological activities (Prachayasittikul et al., 2008; Chen et al., 2008; Pesewu et al., 2008; Turker and Usta, 2008). Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles against

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various diseases (Kumar et al., 2004; Sheeja and Kuttan, 2007; Mukherjee et al., 2007). The rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent intensive for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide (Kaur and Arora, 2009; Mothana et al., 2009; Adedapo et al., 2009). Free radicals which have one or more unpaired electrons (superoxide, hydroxyl, peroxy) are produced in normal or pathological cell metabolism and the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells (Halliwell, 1995; Squadriato and Peyor, 1998; Gulcin et al., 2001). Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. Free radicals or reactive oxygen species (ROS) are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result occasional challenges. These free radicals are the main culprits in lipid peroxidation. Plants congaing bioactive compounds have been reported to possess strong antioxidant properties. In many inflammatory disorders there is excessive activation of phagocytes, production of $O^{2\cdot-}$ OH radicals as well as non free radicals species (H_2O_2) (Gilham et al., 1997), which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and $-OH$ radical formed from $O^{2\cdot-}$ which initiates lipid peroxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by production of mediators and chemotactic factors (Lewis, 1989). The reactive oxygen species are also known to activate matrix metallo proteinase damage seen in various arthritic tissues (Cotran et al., 1994).

Wedelia trilobata (Asteraceae) is a creeping evergreen perennial that roots at the leaf nodes and spreads widely. *W. trilobata* has been historically used for amenorrhea (Lans, 1996), they contain the diterpene (kaurenoic acid), eudesmanolide lactones and luteolin (in leaves and stems) (Lans, 1996; Block et al., 1998). Kaurenoic acid has antibacterial, larvicidal and tripanocidal activity; it is also a potent stimulator of uterine contractions (Block et al., 1998). Luteolin exerts antitumoural, mutagenic and antioxidant effects, has depressant and a stimulant action (Block et al., 1998). Antimicrobial activity was done using *W. trilobata* in other country (Taddei and Rosas-Romero, 1999). Chemical and pharmacological examination of antinociceptive constituents of *Wedelia* sp was done (Block et al., 1998).

The literature survey indicates that no reports are available from India regarding antimicrobial, antioxidant and anti-inflammatory activity of *W. trilobata*. In present study was aimed to examine the total phenolic content and phytochemical analysis of ethanol extract of stem, leaves and flower of *W. trilobata* were screened for antimicrobial, antioxidant and anti-inflammatory properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of

the plant.

MATERIALS AND METHODS

The plant was collected in November 2009 from our college campus (Shridevi Institute of Engineering and Technology, Sira Road, Tumkur, Karnataka, India). The plant was identified by their vernacular names and later it was compared with the herbarium of the Department of Studies in Botany, Manasa Gangothri, University of Mysore, Mysore and Government Ayurvedic College, Mysore, India.

Extract preparation

Plant parts (flower, stem and stem) were air dried at room temperature for 4 weeks to get consistent weight. The dried parts were later ground to powder. Dried parts were used for extract using ethanol separately by microwave method (two-cycle method for 5 min) according to method of Martino et al. (2006). The extracts were filtered using Buckner Funnel and Whatmann No1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. Each extract was resuspended in the solvent ethanol to yield a 50mg/ml stock solution (Taylor et al., 1996; Adedapo et al., 2008).

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, cardiac glycosides, terpenoids, steroids, tannins, phenol, anthroquinone, alkaloids (Obdoni and Ochuko, 2001) and tannins (Kaur and Arora, 2009) was performed as described by the authors. Wagner's and Heger's reagents was used for alkaloid foam test for saponins, Mg-HCl and Zn-HCl for flavonoids, Keller-Killani test for cardiac glycosides, Salkonoski test for terpenoids, acetic anhydride and sulphuric acid for steroids, chloride and gelatin for tannins, ferric chloride for phenol, hexane and diluted ammonia for anthraquinones test. All these experiments were carried out for ethanol extract of dry parts of stem, leaf and flower individually.

Determination of total phenolic content

Total phenolic content (TPC) in extracts was determined by Folin-Ciocalteu's colorimetric method as described by Adedapo et al. (2009b). Extracted solution (0.3 ml in triplicate) was mixed with 1.5 ml of 10% Folin-Ciocalteu's reagent and 1.2 ml of 7.5% (w/v) sodium carbonate. The mixture was kept in the dark for 30 min and absorbance was measured at 765 nm. Quantification was done on the basis of a standard curve of gallic acid. The results were expressed as gallic acid equivalent (GAE), that is, mg gallic acid/100 ml. All tests were performed in triplicate.

Determination of antimicrobial activity

Antimicrobial assay

Bacillus subtilis, *Pseudomonas fluorescens*, *Clavibacter michiganensis* sub sp. *michiganensis*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum* and strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* bacteria were obtained from stock cultures presented at -80°C at Department of Studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of

Studies in Biotechnology and Microbiology, Bangalore University, Gnaana Bharathi, Bangalore, India respectively. Three Gram positive bacteria tested were *B. subtilis*, *C. michiganensis* sub sp. *michiganensis*, *S. aureus* and six Gram negative bacterias tested were *P. fluorescens*, *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, *E. coli*, *P. aeruginosa* and *K. pneumonia*. All bacteria were grown on nutrient agar media.

Fungi (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus flaviceps*, *Alternaria carthami*, *Alternaria helianthi*, *Cercospora carthami*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium verticilloides* and *Nigrospora oryzae*) were obtained from Department of studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangotri, Mysore, Karnataka, India and Department of studies in Microbiology, Bangalore University, Gnaana Bharathi, Bangalore, India respectively. All fungi were grown on potato dextrose agar medium.

Paper disc method

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. (2009) and Adedapo et al. (2008). A swab of the bacteria suspension containing 1×10^8 cfu/ml was spread on to Petri plates containing nutrient agar media. Each extracts were dissolved in ethanol to final concentration of 10 mg/ml. Sterile filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37°C for 24 h. The ethanol served as negative control while the standard streptomycin (10 µg) discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

Determination of antioxidant activity

In order to investigate the antioxidant properties of the examined extracts, ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

FRAP assay

FRAP reagents was freshly prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5 ml FeCl₃ (20 mM) water solution. Each sample (150 µl) (0.5 mg/ml) dissolved in methanol was added in 4.5 ml of freshly prepared FRAP reagent and stirred and after 5 min, absorbance was measured at 593 nm, using FRAP working solution as blank (Szollosi and Szollosi Varga, 2002; Tomic et al., 2009). A calibration curve of ferrous sulfate (100 to 1000 µmol/L) was used and results were expressed in µmol Fe²⁺/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

DDPH radical assay

The effect of different parts ethanolic extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). DPPH solution was freshly prepared by dissolve 24 mg DPPH in 100 ml ethanol, stored at -20°C before use. 150 µl of sample (10 µl sample + 140 µl distilled water) is allowed to react with 2850 µl of DPPH reagent (190 µl reagent + 2660 µl distilled water) for 24 h in the dark condition. Absorbance was measured at 515 nm. Standard curve is linear between 25 to 800 µM ascorbic acid. Results expressed in µm AA/g fresh mass. Additional dilution needed if the DPPH value measured will over the linear range of

the standard curve. Mix 10 ml of stock solution in a solution of 45 ml of methanol, to obtain an absorbance of 1.1 ± 0.02 units at 517 nm using spectrophotometer (Katalinic et al., 2006). All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to formula of Yen and Duh (1994):

$$\% \text{ inhibition} = \frac{[\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}]}{\text{Abs}_{\text{control}}} \times 100$$

where Abs_{control} is the absorbance of the DPPH radical+ ethanol, Abs_{sample} is the absorbance of DPPH radical+ sample extract/standard.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

Methods of Mizushima and Kobayashi (1968) and Sakat et al. (2010) followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = \frac{[\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}]}{\text{Abs}_{\text{control}}} \times 100$$

where Abs_{control} is the absorbance of the DPPH radical+ ethanol, Abs_{sample} is the absorbance of DPPH radical+ sample extract/standard.

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline (Sadique et al., 1989; Saket et al., 2010).

Heat induced hemolytic

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned above (Shinde et al., 1999; Saket et al., 2010).

Protein inhibitory action

The test was performed according to the modified method of Oyedepo et al. (1995) and Sakat et al. (2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The

Table 1. Determination of total phenolic content from ethanolic extract of leaf, stem, flower of *W. trilobata*.

Sample	TPC (mg gallic acid/100 ml)	Yield (g/mg dry parts)
Ethanol extract		
Leaf	9.41±0.03 ^a	9.3
Stem	6.71±0.07 ^b	5.4
Flower	4.88±0.07 ^c	5.1

Repeated the experiments three times for each replicates. According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at $P \leq 0.05$, SE-standard error of the mean.

reaction mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups ($p < 0.05$). Means between treatment groups were compared for significance using Duncan's new Multiple Range post test.

RESULTS

Total phenol contents and antioxidant activity

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent and expressed in terms of mg gallic acid equivalent (GAE)/ 100 ml extract. The more TPC was observed in leaf (9.41) followed by stem (6.71) and flower (4.88) (Table 1).

The antioxidant activity of the ethanol crude extract and its various fractions, as measured by the ability to scavenge DPPH free radicals, was compared with the standards/ ascorbic acid and butylated hydroxytoluene (BHT). It was observed that ethanol extract of the leaf of *W. trilobata* had higher activity than that of stem and flower. At a concentration of 0.1 mg/ml, the scavenging activity of ethanol extract of the stem and flower reached 82.64 and 55.41% respectively while at the concentration, that of leaf was 86.17%. Though the DPPH radical scavenging abilities of the extract were less than those of ascorbic acid (98%) and BHT (97.8%) at 0.1 mg/ml, the study showed that the extracts have the proton donating ability and could serve as free radical inhibitors or scavenging, acting possibly as primary antioxidants (Figure 1).

The reducing ability of the extract was in the range of 172.32 to 630.72 $\mu\text{m Fe (II)}/\text{mg}$ (Table 2). The antioxidant potentials of the ethanol extracts of leaf and stem of *W. trilobata* were estimated from their ability to reduce

TPRZ-Fe (III) complex to TPTZ-Fe (II). The FRAP values for the ethanol extract of leaf and stem were significantly lower that of ascorbic acid but higher that of BHT.

Phytochemical analysis

The phytochemical screening showed that the different extracts of *W. trilobata*, the tannin, flavonoids, terpenoids, phenol and saponins were present in all extracts (leaf, stem and flower). The steroids, alkaloids, anthraquinones were absent in all the extracts. However, some phytochemicals, cardiac glycosides was present in only flower extract (Table 3).

Antimicrobial assay

The antimicrobial activities of ethanolic extract of leaf, stem, flower of *W. trilobata* gave different zones of inhibition on the organisms tested (Table 4). The ethanolic stem extract inhibited the growth of all most all the bacteria isolates but all the extracts did not showed any significant effect on fungal isolates. The leaf extract showed more potent against *P. aeruginosa*, *K. pneumoniae*, *P. fluorescens*, *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, moderately inhibited the *E.coli*, *C.michiganensis* sub sp. *michiganensis* but less activity was observed on *S. aureus*. All the extracts exhibited less activity on all species of *Fusarium* and *Aspergillus*. Whereas all the extracts did not showed any effect on *Alternaria* species, *Cercospora carthami* and *Nigrospora oryzae* and all these pathogens have showed resistance against all the extracts which was tested.

Anti inflammatory properties

Inhibition of albumin denaturation

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. It was effective

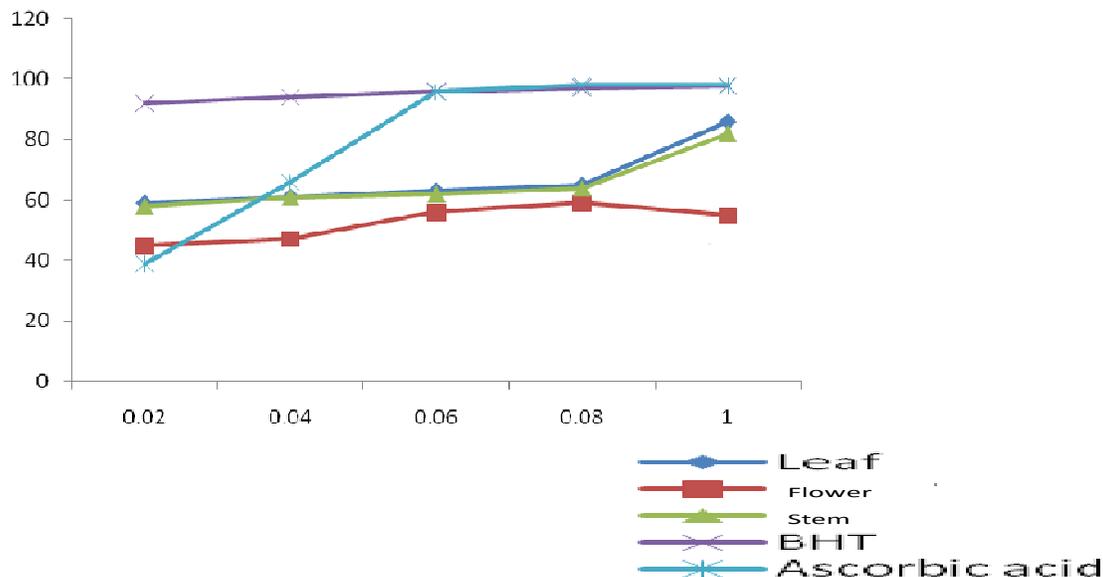


Figure 1. DPPH scavenging activities of the ethanol extracts of the flower leaves and stem of *W. triloba*.

Table 2. Total antioxidant (FRAP) activities of ethanol extracts of the leaves, stem and flower.

Extracts	FRAP
Ethanol extract	
Leaf	630.72±0.06 ^a
Stem	508.81±0.06 ^a
Flower	172.32±0.05 ^{ab}
Ascorbic acid	1648.52±0.06 ^a
BHT	64.84±0.06 ^b

Repeated the experiments three times for each replicates. According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at $P \leq 0.05$, SE-standard error of the mean.

Table 3. Phytochemical analysis of ethanolic extract of different plant parts.

Tests	Leaf	Stem	Flower
Tannin	+ve	+ve	+ve
Steroids	-ve	-ve	-ve
Cardioglycosides	-ve	-ve	+ve
Flavonoids	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve
Phenol	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Anthraquinones	-ve	-ve	-ve

+ve: positive, -ve: negative

in inhibiting heat induced albumin denaturation (Table 5). Maximum inhibition 87.14% was observed from leaf

extract followed by stem (86.76%) and flower (61.63%). Aspirin, a standard anti-inflammation drug showed the

Table 4. *In vitro* inhibition assay from ethanolic extracts.

Species	Stem	Flower	Leaves
Bacterial pathogens			
<i>E. coli</i>	++	+	+++
<i>P. aeruginosa</i>	+++	+	++
<i>S. aureus</i>	+	+++	++
<i>K. pneumonia</i>	+++	++	++
<i>P. fluorescens</i>	+++	++	++
<i>C. michiganensis</i> sub sp. <i>michiganensis</i>	++	+	++
<i>X. oryzae</i> pv. <i>oryzae</i>	+++	+++	+
<i>X. axanopodis</i> pv. <i>malvacearum</i>	+++	++	++
Fungal pathogens			
<i>Aspergillus flavus</i>	+	+	+
<i>A. niger</i>	+	+	+
<i>A. nidulans</i>	+	+	+
<i>A. flaviceps</i>	+	+	+
<i>Alternaria carthami</i>	-	-	-
<i>A. helianthi</i>	-	-	-
<i>Cercospora carthami</i>	-	-	-
<i>Fusarium solani</i>	+	+	+
<i>F. oxysporum</i>	+	+	+
<i>F. verticilloides</i>	+	+	+
<i>Nigrospora oryzae</i>	-	-	-

+++ average, += minimum activity, -= No activity. Repeated the experiments three times for each replicates.

Table 5. Effect of ethanol and water extracts of *Wedalia trilobata* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition.

Test sample	Albumin denaturation	Membrane stabilization	Proteinase inhibition
Ethanol extract			
Leaf	87.14±0.006 ^a	78.11±0.03 ^a	84.19±0.05 ^a
Stem	86.76±0.006 ^a	74.17±0.03 ^a	81.84±0.05 ^a
Flower	61.63±0.006 ^c	58.74±0.05 ^b	67.17±0.03 ^b
Aspirin (200 µg/ml)	75.89±0.006 ^b	85.92±0.03 ^a	92.87±0.05 ^a

Repeated the experiments three times for each replicates. According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at $P \leq 0.05$, SE-standard error of the mean.

maximum inhibition 76.69% at the concentration of 200 µg/ml.

Membrane stabilization test

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different ethanolic extract of *W. trilobata*. All the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect. This effect may possibly inhibit the release of

lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree (Table 5). The maximum inhibitions 78.11% from leaf extract followed by stem (74.17%) and flower (58.74%). The aspirin standard drug showed the maximum inhibition 85.92%.

Proteinase inhibitory activity

The *W. trilobata* ethanolic extract exhibited significant antiproteinase activity from different parts. The maximum inhibition was observed from leaf ethanolic extract

(84.19%), in decreasing order was stem (81.84%) and flower ethanolic extract (67.17%). The standard aspirin (92.87%) drug showed the maximum proteinase inhibitory action (Table 5).

DISCUSSION

In recent years, the search for phytochemicals possessing antioxidant, antimicrobial and anti-inflammatory properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc (Halliwell, 1996). Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes (Berahou et al., 2007).

Results of our findings confirmed the use of *W. trilobata* as traditional medicine. We found strong antioxidants, antimicrobial and anti-inflammatory activities specifically in the ethanolic leaf and stem extracts of *W. trilobata*. High TPC values found in ethanolic leaf and stem extracts (9.41 and 6.81 mg GAE/100 ml) imply the role of phenolic compounds in contributing these activities. Plant phenolic compounds have been found to possess potent antioxidants (Adedapo et al., 2009b; Adesegun et al., 2009; Lai et al., 2010), antimicrobial (Kaur and Arora, 2009; Alcaraz et al., 2000; Lai et al., 2010) and anti-inflammatory activity (Sakat et al., 2010; Roy et al., 2010; Garg et al., 2010).

The flavonoids from plant extracts have been found to possess antioxidants, antimicrobial and anti-inflammatory properties in various studies (Lin et al., 2008; Lopez-Lazaro, 2009; Yoshida et al., 2009; Amaral et al., 2009). The presence of terpenoids have shown as antimicrobial (Singh and Singh, 2003), antioxidant (Grassman, 2005) and anti-inflammatory properties (Neukirch et al., 2005).

Strong presence of tannins in all extracts may explain its potent bioactivities as tannins are known to possess potent antimicrobial activities (Kaur and Arora, 2009), antioxidants (Zhang and Lin, 2008), and anti-inflammatory properties (Fawole et al., 2010). The saponins have already shown as antimicrobial activity (Mandal et al., 2005), antioxidant activity (Gulcin et al., 2004) and anti-inflammatory activity (Gepdireman et al., 2005).

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability

to thermally induced protein denaturation (Mizushima and Kobayashi, 1968). Similar results were observed from many reports from plant extract (Sakat et al., 2010). The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). The precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the *W. trilobata* produced this effect surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins (Shinde et al., 1999).

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995). Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the antioxidant and anti-inflammatory activities of many plants. Hence, the presence of bioactive compounds in the ethanolic extract of different parts of *W. trilobata* may contribute to its, antimicrobial, antioxidant and anti-inflammatory activity.

The present investigation has shown that the leaf and stem extract of *W. trilobata* have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The ethanol leaf and stem extract fractions showed significantly antimicrobial activity against all Gram-positive and Gram-negative bacteria and different fungi tested. Strong antioxidant and anti-inflammatory properties were confirmed in the ethanol extract fractions. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols and Saponins. The antioxidant activity and anti-inflammatory activity was comparable with standard ascorbic acid, BHT and aspirin. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial, antioxidant and anti-inflammatory agent from *W. trilobata* plant. This medicinal plant by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antimicrobial, antioxidant and anti-inflammatory drugs.

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