Journal of Medicinal Plants Research Vol. 5(24), pp. 5718-5729, 30 October 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

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

Govindappa M.[1]*, Naga Sravya S.[1], Poojashri M. N.[1], Sadananda T. S.[1], Chandrappa C. P.[1], Gustavo Santoyo[2], Sharanappa P.[3] and Anil Kumar N. V.[4]

[1]Department of Biotechnology, Shridevi Institute of Engineering and Technology, Sira Road, Tumkur-572 106, Karnataka, India.
[2]IIQB-UMSNH, Edificio A1 (B5), Ciudad Universitaria, Morelia, Michoacan, Mexico, C.P. -58030, Mexico.
[3]Department of Studies in Biosciences, University of Mysore, Hemagangothri, Hassan -573 220, India.
[4]Department of Chemistry, Manipal Institute of Technology, Manipal University, Manipal -576 104, India.

Accepted 13 September, 2011

The aim of the study was to evaluate antimicrobial, antioxidant and anti-inflammatory activity of dry and fresh parts of leaf, stem and flower from the water extract of *Wedelia trilobata.* The antimicrobial activity of water extracts of fresh and dry parts against 9 different strains of bacteria and 11 different species of fungi were determined using standard method (paper disc method). The fresh parts water extracts showed that, leaf and flower extracts were most potent inhibiting all isolates of with different zones of inhibition but did not inhibited the growth of fungi tested. All the extracts have only moderately inhibited the all fungi. The minimum microbial concentration (MMC) of the active extract was observed from fresh part extracts of leaf, flower and stem ranged from 0.4 to 5.0 mg/ml for the sensitive bacteria. In case of fungi, the minimum inhibitory concentration (MIC) of the active extracts ranged from 2.4 to 6.0 mg/ml. Together, these data suggest that the *W. trilobata* fresh parts extracts analyzed are potential antimicrobial candidates with a broad range of activity. Phytochemical screening of extracts showed the presence of tannins, cardiac glycosides, flavonoids, terpenoids, phenols, saponins and coumarins. Leaf and flower water have showed highest total phenolic content. In 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) method, the leaf and flower had showed free radical inhibition of 86, 83 and 1623.21, 1611.26, respectively and they also showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization with 89.61 and 86.81 and 78.82, 76.65 g/ml, respectively. Proteinase activity was also significantly inhibited by the leaf (83.91 g/ml) and flower (81.17 g/ml). From the result, it is concluded that phytochemicals present in the *W. trilobata* extract may be responsible and can be used as antimicrobial, antioxidant and anti-inflammatory agent.

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

INTRODUCTION

Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto-medicine for the treatment of disease (Iwu et al., 1999). It is estimated that plant materials are present in, or have provided the models for 50% Western drugs (Rodders et al., 1996). The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments (Bandow et al., 2003). Many plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today, the

*Corresponding author. E-mail: dravidateja07@yahoo.co.in. Tel: +91-9686114847 or +91-816-2212626. Fax: +91-816-2212629.

search for natural compounds rich in antimicrobial, antioxidant and anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic disorders (cancer, diabetes, arthritis, hypertension etc). Natural products derived from plants offer a new source of biological that may have a great impact overall human health (Baladrin et al., 1985). The rapid emergence of multiple drug resistant strains of pathogens to current antimicrobial agents has generated an urgent intensive search for new antibiotics from medicinal plants. 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). Many medicinal plants have been screened extensively for their anti-microbial potential worldwide (Kaur and Aroara, 2009).

Free radicals are a major cause of oxidative stress that may lead to DNA strand breakage, gene mutation and DNA-DNA and DNA-protein cross links. Free radicals are known to be a product of normal metabolism. When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and H_2O_2 are generated (Halliwell and Gutteridge, 1989). ROS are involved in an organism's vital activities including phagocytosis, regulation of cell proliferation, intracellular signaling and synthesis of biologically active compounds (Miquel and Romano-Bosca, 2004).

ROS have been implicated in several diseases including carcinogenesis, malaria, heart diseases, arteriosclerosis, diabetes and many other health problems related to ageing (Honda et al., 2004; Espin et al., 2000). The role of ROS in the etiology and progression of several clinical manifestations has led to the suggestion that the antioxidants can be beneficial as prophylactic agents. Nevertheless, all aerobic organisms, including humans, have antioxidant defenses that protect against oxidative harm and repair damaged molecules. However, the natural antioxidant mechanisms can be insufficient, the supply of antioxidants through dietary ingredients, is of great interest for a healthy life (Honda et al., 2004; Greenwald et al., 2001).

In many inflammatory disorders there is excessive activation of phagocytes, production of 0_2-, OH radicals as well as non free radicals species (H_20_2) (Gilham, 1997), which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and –OH radical formed from O_2- 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 metello proteinase damage seen in various arthritic tissues (Cotran et al., 1994). Drugs from plant origin are relied upon by 80% of the world's population. In India, the use of herbal drugs is an important component of the traditional system of medicine. Knowing the fact that several diseases (diabetes, obesity etc.) have been treated by the administration of plant extracts from medicinal plants (Lans, 1996), the present investigation was aimed at evaluating the antimicrobial, antioxidant and anti-inflammatory potential of water extract of three different parts (leaf, stem and flower) of *Wedelia trilobata*. *W. trilobata* has been historically used for amenorrhea (Block et al., 1998), they contain the diterpene (kaurenoic acid), eudesmanolide lactones and luteolin (in leaves and stems). Kaurenoic acid has antibacterial, larvicidal and tripanocidal activity; it is also a potent stimulator of uterine contractions (Obdoni and Ochuko, 2001).

The literature survey indicates that, Taddei and Rosas-Romero (1999) and Maldini et al. (2009) have reported on antimicrobial and anti-inflammatory activity of *W. trilobata*. In India, no reports are available regarding antimicrobial, antioxidant and anti-inflammatory activity of *W. trilobata*. 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 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. 100 g of fresh and dried samples were extracted with distilled water (60 to 80°C, 200 ml for both fresh and dry parts of leaves, stem and flower) for 2 days in water both with a shaking attachment. The extract was lyophilized under 5 µm Hg pressure and stored at -20°C. The experimental were carried out using an appropriate amount of lyophilized material.

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, cardiac glycosides, terpenoids, steroids, tannins, phenol, anthroquinone, alkaloids (Adedapo et al., 2009) and tannins (Kaur and Aroara, 2009) was performed as described by the authors for all extracts. 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 water extract of fresh and dry parts of stem, leaf and flower separately.

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. (2009). 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, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia* 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 Microbiology and Biotechnology, Bangalore University, Gnana Bharathi, Bangalore, India, respectively. Three Gram positive bacteria tested were *B. subtilis*, *C. michiganensis* sub sp. *michiganensis*, *S. aureus* and six Gram negative bacterial 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 Gangothri, Mysore, Karnataka, India and Department of Studies in Microbiology and Biotechnology, Bangalore University, Gnana 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). A swab of the bacterial and fungal suspension containing 1×10^3 and 1×10^3 cfu/ml was spread on to Petri plates containing nutrient agar and potato dextrose media. Each extracts were dissolved in water 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 water served as negative control while the standard chloromphenicol (10 µg) and copper oxychloride for bacteria and fungi respectively and 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 for each experiments.

Minimal antimicrobial concentration

The minimal microbial concentration (MMC) of the active extracts was determined for bacteria and fungi as described by Verastegui et al. (2008). Activated bacterial cultures (1×10^3 CFU/ml) were grown in tubes containing 3 ml of NA broth in the presence of fresh and dry extracts leaf, stem and flower of *W. trolibata* were added in 0.1 mg/ml increments separately. Cultures were incubated for 24 h at 37°C and microbial survival was determined by plate count using NA. The MMC was defined as the lowest concentration of the extract that prevented visible microbial growth on agar plate at the conclusion of the incubation period. The minimal inhibitory concentration MIC for fungi was determined using the method as previously described by Verastegue et al. (1996). An appropriate amount of each extract was aseptically mixed with the sterile PDA to reach a final concentration of 1 to 10 mg/ml in 0.5 mg/ml increments. Each concentration was poured into one section of a Petri plates that have divided into thirds and each section was streaked uniformly with 1×10^3 fungi spores or infective mycelia particles. Fungi were incubated for ten days at room temperature (24±2°C). The MIC was defined as the lowest concentration of extract that prevented the growth of the fungi as evaluated by microscope at 10x magnification. Each assay was replicated at least three times.

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 $FeCl_3$ (20 mM) water solution. Each sample (150 L) (0.5 mg/ml) dissolved in water 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). A calibration curve of ferrous sulfate (100 to 1000 mol/L) was used and results were expressed in mol Fe^{2+}/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

DDPH radical assay

The effect of endophytic 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 water, 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 water, to obtain an absorbance of 1.1±0.02 units at 517 nm using spectrophotometer. 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):

$$\% \text{ inhibition} = [\{Abs_{control} - Abs_{sample}\}/Abs_{control}] \times 100,$$

***In vitro* anti-inflammatory activity**

Inhibition of albumin denaturation

Methods of 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:

% inhibition= [{Abs $_{control}$- Abs $_{sample}$}/Abs $_{control}$] x 100,

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 (Sakat 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 in the foregoing (Sakat et al., 2010).

Protein inhibitory action

The test was performed according to the modified method of Sakat et al. (2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH7.4) and 1 ml test sample of different concentrations. The 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.

Estimation of coumarin(s) (esuletin and scopoletin)

Esculetin

Different plant extracts were subjected to acid wash in the presence of neutral sand and 5ml of distilled water for 15 min in pestle and mortar, followed by centrifuge at 5000 rpm for 15 min and make up the collected supernatant volume to 5 ml using distilled water. Absorbance is read at 340 nm (Tamma and Miller, 1985).

Scopoletin

Different plant extracts are obtained by microwave oven method (2 cycles). Absorbance is read at 605 nm (Solich et al., 1995).

Statistical analysis

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

RESULTS

Antimicrobial assay

The antimicrobial activities of water extract of leaf, stem, flower of *W. trilobata* gave different zones of inhibition on the organisms tested. The water fresh leaf extract inhibited the growth of all the bacteria isolates but all the extracts did not show any similar significant effect on fungal isolates. The leaf extract showed more potent against *Pseudomonas aeruginosa, E. coli, P. fluorescens*, *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, moderately inhibited the, *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*. All most all extracts that is dry or fresh parts extracts had showed similar activity on tested microorganisms, where as standards chloromphenicol for bacteria and copper oxychloride for bacteria have shown significant inhibition on all bacteria and fungi, respectively (Table 1).

The MMC and MIC of fresh parts water extracts of leaf, flower and stem of *W. trilobata* against bacteria ranged from 0.4 to 4.5 mg/ml and dry parts extracts ranged from 0.4 to 5.0 mg/ml (Table 2). The leaf extract was more active against all bacterial strains. The MMC for bacteria and the MIC for fungi of *W. trilobata* fresh parts extracts ranged from 2.4 to 6.0 mg/ml for all the organisms tested (Table 3). The different parts of water extracts of leaf, flower and stem analysed here are potential candidates for broadly active antimicrobial compounds. Although the antimicrobial activity of the whole plant extracts are low compared to antibiotics or fungicide (Tables 2 and 3), purification of the active natural compounds could derive a metabolite more active.

Phytochemical analysis

The phytochemical screening showed that the different extracts of *W. trilobata*, the tannin, cardiac glycosides, terpenoids, flavonoids, saponins were present in all fresh and dry extracts of leaf, stem and flower. The steroids, alkaloids and anthraquinones were absent in all the extracts (Table 4).

Total phenol contents and antioxidant activity

Total phenolic content (TPC) was determined using the Folin-ciocalteau reagent and expressed in terms of mg gallic acid equivalent (GAE)/100 ml extract. The more TPC was observed in dry leaf extract (9.83) followed by flower (9.22) and stem (3.87) (Table 5). The water fresh plant extracts of leaf (9.96), flower (9.52) and stem (4.14) had showed highest TPC.

The antioxidant activity of the water crude extract measured by the ability to scavenge DPPH free radicals was compared with the standards/ ascorbic acid and butylated hydroxytoluene (BHT). It was observed that fresh water 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 water extract of fresh parts of stem and flower reached 82.64 and 55.41%, respectively while at the concentration, that of leaf was 86.17% (Figure 1). The dry part

Table 1. *In vitro* inhibition assay from water extracts.

Species	Stem		Flower		Leaves		Chloromphenicol
	Fresh	Dry	Fresh	Dry	Fresh	Dry	
Bacterial pathogens							
E. coli	8±1^{a}	8±1^{a}	16±2^{a}	16±2^{a}	15±2^{b}	15±2^{a}	20±2^{c}
Bacillus subtilis	8±1^{a}	8±1^{a}	16±2^{a}	15±2^{b}	14±2^{c}	14±2^{b}	21±2^{b}
Pseudomonas aeruginosa	3±1^{b}	2±1^{c}	16±2^{a}	15±2^{b}	16±2^{a}	15±2^{a}	21±2^{b}
Staphylococcus aureus	2±1^{c}	3±1^{b}	2±1^{c}	2±1^{e}	2±1^{f}	2±1^{d}	18±2^{d}
Klebsiella pneumonia	NI	NI	NI	NI	NI	NI	18±2^{d}
Pseudomonas fluorescens	2±1^{c}	2±1^{c}	6±1^{b}	6±1^{d}	6±1^{e}	9±1^{c}	21±2^{b}
Clavibacter michiganensis sub sp. *michiganensis*	2±1^{c}	2±1^{c}	2±1^{c}	2±1^{e}	8±1^{d}	2±1^{d}	16±2^{e}
Xanthomonas oryzae pv. *oryzae*	2±1^{c}	2±1^{c}	16±2^{a}	8±1^{c}	15±2^{b}	14±2^{b}	16±2^{e}
Xanthomonas axanopodis pv. *malvacearum*	2±1^{c}	2±1^{c}	6±1^{b}	6±1^{d}	6±1^{e}	14±2^{b}	22±2^{a}
Fungal pathogens							**Copper oxychloride**
Aspergillus flavus	2±1^{a}	2±1^{a}	6±1^{c}	6±1^{d}	8±1^{b}	8±1^{a}	21±2^{a}
Aspergillus niger	2±1^{a}	2±1^{a}	6±1^{c}	6±1^{d}	8±1^{b}	8±1^{a}	21±2^{a}
Aspergillus nidulans	2±1^{a}	2±1^{a}	8±1^{b}	7±1^{c}	8±1^{b}	8±1^{a}	21±2^{a}
Aspergillus flaviceps	2±1^{a}	2±1^{a}	6±1^{c}	7±1^{c}	8±1^{b}	8±1^{a}	21±2^{a}
Alternaria carthami	NI	NI	NI	NI	NI	NI	19±2^{c}
Alternaria helianthi	NI	NI	NI	NI	NI	NI	21±2^{a}
Cercospora carthami	NI	NI	NI	NI	NI	NI	21±2^{a}
Fusarium solani	2±1^{a}	2±1^{a}	14±2^{a}	15±2^{a}	15±2^{a}	6±1^{b}	19±2^{c}
Fusarium oxysporum	2±1^{a}	2±1^{a}	14±2^{a}	15±2^{a}	15±2^{a}	6±1^{b}	19±2^{c}
Fusarium verticilloides	2±1^{a}	2±1^{a}	14±2^{a}	14±2^{b}	14±2^{a}	6±1^{b}	20±2^{b}
Nigrospora oryzae	NI	NI	NI	NI	NI	NI	20±2^{b}

NI = No Inhibition. 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.

extracts of leaf (83.12%), flower (54.26%) and stem (79.33%) showed low scavenging activity as compared with fresh plant parts (Figure 2). 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.

The reducing ability of the fresh plant extracts was in the range of 773.32 to 16230.21 µm Fe (II)/mg (Table 6). The antioxidant potentials of the water fresh part extracts of leaf (1623.21±0.06) and flower (1611.26±0.06) and dry extracts of leaf (1432.64±0.08) and flower (1368.57±0.09) *W. trilobata* were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The FRAP values for the water extract of leaf and stem were significantly lower than that of ascorbic acid but higher than that of BHT.

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 in inhibiting heat induced albumin denaturation (Table 7). Maximum inhibition 89.61±0.06 was observed from fresh leaf extract followed by flower (86.81±0.06) and stem (51.14±0.08). In dry parts water extract also inhibited the albumin denaturation, here also the leaf extracts (86.26±0.06) stood first followed by flower (48.22±0.06) and stem (82.11±0.07). Aspirin, a standard anti-inflammation drug showed the maximum inhibition 76.89% 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 water 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 7). The maximum inhibition was recorded 78.82±0.06 from leaf extract followed by flower (76.65±0.05) and stem (52.31±0.06) from fresh parts extracts. In dry parts extracts, the leaf (74.63±0.06) has shown highest membrane stabilization activity followed by flower (72.46±0.04) and stem (50.72±0.06). The aspirin standard drug showed the maximum inhibition 85.92%.

Proteinase inhibitory activity

The *W. trilobata* water extract exhibited significant antiproteinase activity from different parts. The maximum inhibition was observed from fresh leaf water extract (83.91±0.03), in decreasing order was fresh flower (81.17±0.03) and fresh stem (64.84±0.06) water

Table 2. Minimal antimicrobial activity of water extract of leaf, stem and flower of *W. trilobata*.

Extracts	Ec	Pa	Sa	Kp	Pf	Cmm	Xoo	Xam	Bs
Fresh parts									
Leaf	0.4±0.3^{b}	1.6±0.2^{c}	1.5±0.2^{c}	NI	1.4±0.2^{f}	1.9±0.4^{d}	3.6±0.4^{d}	2.5±0.4^{d}	0.5±0.3^{b}
Stem	1.6±0.02^{a}	2.2±0.5^{b}	2.3±0.5^{a}	NI	2.8±0.4^{b}	NI	4.3±0.5^{b}	4.5±0.5^{b}	1.5±0.2^{a}
Flower	0.5±0.3^{b}	1.5±0.2^{c}	1.6±0.2^{b}	NI	1.7±0.2^{d}	3.3±0.4^{b}	1.8±0.02^{e}	2.5±0.4^{d}	0.5±0.3^{b}
Dry parts									
Leaf	0.4±0.3^{b}	1.5±0.2^{c}	1.5±0.2^{c}	NI	1.6±0.2^{e}	3.1±0.5^{c}	4.1±0.4^{c}	3.0±0.5^{c}	0.5±0.3^{b}
Stem	1.6±0.02^{a}	2.6±0.6^{a}	2.3±0.5^{a}	NI	3.1±0.5^{a}	NI	4.8±0.5^{a}	5.0±1.2^{a}	1.5±0.2^{a}
Flower	0.6±0.3^{b}	1.5±0.2^{c}	1.5±0.2^{c}	NI	1.8±0.2^{c}	3.5±0.4^{a}	3.6±0.4^{d}	3.0±0.5^{c}	0.5±0.3^{b}
Chloromphenicol	0.02±0.02^{d}	0.02±0.02^{d}	0.02±0.02^{d}	0.04±0.02	0.02±0.02^{g}	0.02±0.02^{e}	0.02±0.02^{f}	0.02±0.02^{e}	0.02±0.02^{c}

NI-No inhibition, According to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at P≤0.05, SE-standard error of the mean. Ec-*E. coli,* Pa-*, Pseudomonas aeruginosa*, Sa- *Staphylococcus aureus*, Kp-*Klebsiella pneumonia*, Pf-*Pseudomonas fluorescens*, Cmm-*Clavibacter michiganensis* sub sp. *michiganensis*, Xoo-*Xanthomonas oryzae* pv. *oryzae*, Xam- *Xanthomonas axanopodis* pv. *malvacearum* and Bs-*Bacillus subtilis*.

Table 3. Minimal antimicrobial activity of water extract of leaf, stem and flower of *W. trilobata*.

Extracts	Af	An	Anid	Afla	Ac	Ah	Cc	Fs	Fo	Fv	No
Fresh parts											
Leaf	2.6±0.3^{c}	2.6±0.3^{e}	2.8±0.3^{c}	2.8±0.3^{f}	NI	NI	NI	3.2±0.5^{f}	3.5±0.6^{c}	3.5±0.5^{d}	NI
Stem	3.3±0.4^{b}	3.5±0.5^{b}	3.4±0.4^{b}	3.6±0.3^{d}	NI	NI	NI	5.1±0.8^{b}	4.5±0.7^{b}	5.0±0.9^{b}	NI
Flower	2.6±0.3^{c}	2.8±0.4^{d}	3.1±0.3^{b}	4.9±0.3^{b}	NI	NI	NI	3.4±0.4^{e}	3.5±0.6^{c}	3.5±0.5^{d}	NI
Dry parts											
Leaf	2.6±0.3^{c}	2.4±0.3^{f}	2.7±0.3^{c}	3.1±0.3^{e}	NI	NI	NI	3.6±0.4^{d}	3.5±0.6^{c}	4.0±0.6^{c}	NI
Stem	3.6±0.4^{a}	3.8±0.5^{a}	4.2±0.5^{a}	4.1±0.3^{c}	NI	NI	NI	6.0±1.2^{a}	5.0±1.1^{a}	6.0±1.1^{a}	NI
Flower	2.6±0.3^{c}	3.1±0.4^{c}	3.3±0.4^{b}	5.6±0.3^{a}	NI	NI	NI	4.0±0.6^{c}	3.5±0.6^{c}	4.0±0.6^{c}	NI
Copper oxychlorode	0.03±0.03^{d}	0.03±0.03^{f}	0.03±0.03^{d}	0.03±0.3^{g}	0.05±0.04	0.03±0.03	0.05±0.04	0.03±0.03^{g}	0.03±0.03^{d}	0.03±0.03^{e}	0.05±0.04

NI- No inhibition. According to Duncan's multiple range test (DMRT), values followed by different subscripts are significantly different at P≤0.05, SE-standard error of the mean. Af- *Aspergillus flavus,* An-*Aspergillus niger,* Anid-*Aspergillus nidulans,* Afla-*Aspergillus flaviceps,* Ac- *Alternaria carthami,* Ah- *Alternaria helianthi,* Cc-*Cercospora carthami,* Fs- *Fusarium solani,* Fo- *Fusarium oxysporum,* Fv-*Fusarium verticilloides* and No- *Nigrospora oryzae*.

extracts. In dry parts water extracts, the leaf (79.33±0.06) extracts flower (77.55±0.05) and stem (64.84±0.06). The standard drug aspirin have showed the maximum proteinase inhibitor activity is 92.83±0.03 (Table 7).

Identification of coumarins in the water extract of leaf, stem and flower of *W. trilobata*

The leaf water extract was showed presence of coumarins (esculetin and scopoletin) at higher concentration in spectro-photometrically, in decreasing order was flower and stem of those two coumarin(s) in fresh parts extracts. In present investigation, we followed the method of

Table 4. Phytochemical analysis of water extract of different plant parts.

Tests	Leaf		Stem		Flower fresh	Dry
	Fresh	Dry	Fresh	Dry		
Tannin	+ve	+ve	+ve	+ve	+ve	+ve
Steroids	-ve	-ve	-ve	-ve	-ve	-ve
Cardiacglycosides	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
Phenol	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	+ve	+ve	+ve
Anthraquinones	-ve	-ve	-ve	-ve	-ve	-ve

+ve: positive, -ve: negative. Repeated the experiments three times for each replicates.

Table 5. Determination of total Phenolic content from water extract of leaf, stem, flower of *W. trilobata.*

Sample		TPC (mg gallic acid/100 ml)	Yield (g/mg dry parts)
Leaf	Dry	9.83 ± 0.03^{b}	1.8
	Fresh	9.96 ± 0.03^{a}	2.2
Stem	Dry	3.87 ± 0.09^{f}	5.8
	Fresh	4.14 ± 0.09^{e}	4.4
Flower	Dry	9.22 ± 0.03^{d}	2.6
	Fresh	9.52 ± 0.03^{c}	2.9

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.

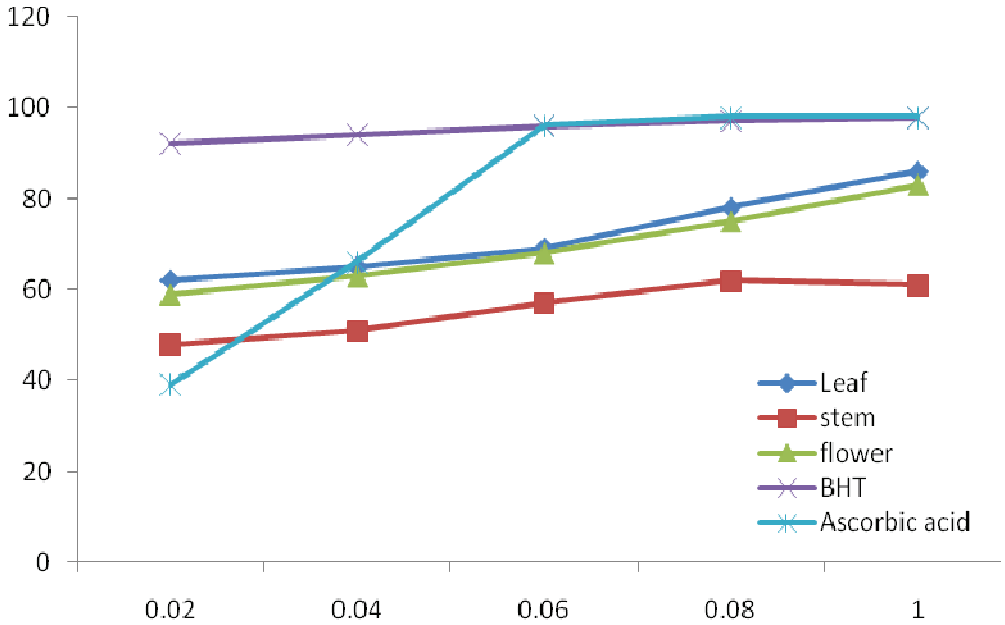

Figure 1. DPPH scavenging activities of the water extracts of fresh parts of leaf, stem and flower of *Wedelia trilobata.*

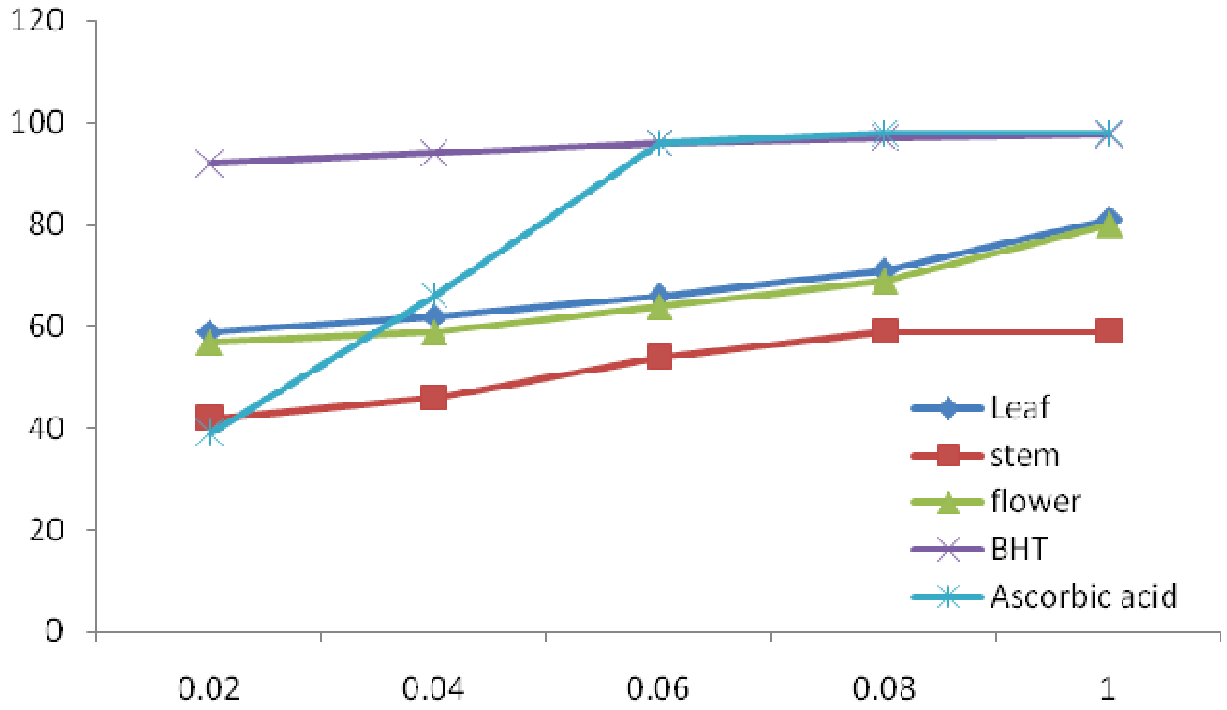

Figure 2. DPPH scavenging activities of the water extracts of dry parts of leaf, stem and flower of *Wedelia trilobata*.

Table 6. Total antioxidant (FRAP) activities of water extracts of the leaves, stem and flower.

Extracts		FRAP
Leaf	Fresh	1623.21±0.06[a]
	Dry	1432.64±0.08[d]
Stem	Fresh	810.14±1.2[f]
	Dry	773.32±1.4[g]
Flower	Fresh	1611.26±0.06[b]
	Dry	1368.57±0.09[e]
Ascorbic acid		1648.52±0.06[c]
BHT		64.84±1.5[h]

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.

Martino et al. (2006) have reported that microwave oven assisted extraction is suitable to extract coumarin from the sample. Esculetin was yielded more in percentage in fresh leaf extract (2.6), followed by flower (2.3) and stem (1.3) (Table 8).

The dry parts water extracts also shown highest percentage, the decreasing order is leaf (2.7), flower (2.4) and stem (1.1). As compared to fresh parts extracts the esculetin was observed more dry parts extracts. More percentage of scopoletin identified in fresh parts extracts leaf (3.3), flower (3.3) and stem (2.1), where is in dry parts water extracts of leaf (2.8) highest scopoletin followed by flower (2.3) and stem (1.6) (Table 9). The fresh parts water extracts yielded the more scopoletin compared to dry parts extracts.

DISCUSSION

In recent years, the search for phytochemicals possessing antioxidant, antimicrobial and antiinflammatory properties have been on the rise due to their potential

Table 7. Effect of water extracts of *Wedelia trilobata* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition.

Test sample	Albumin denaturation	Membrane stabilization	Proteinase inhibition
Fresh extract			
Leaf	89.61±0.06[a]	78.82±0.04[b]	83.91±0.03[b]
Stem	51.14±0.08[e]	52.31±0.06[d]	64.84±0.06[d]
Flower	86.81±0.06[b]	76.65±0.05[b]	81.17±0.03[c]
Dry extract			
Leaf	86.26±0.06[b]	74.63±0.06[c]	79.33±0.06[c]
Stem	48.22±0.07[f]	50.72±0.06[d]	64.84±0.06[d]
Flower	82.11±0.06[c]	72.46±0.04[d]	77.55±0.05[c]
Aspirin (200 µg/ml)	75.89±0.06[d]	85.92±0.02[a]	92.83±0.03[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.

Table 8. Esculetin identification in water plant extracts of *Wedelia trilobata* by spectrophotometric method.

Sample	OD at 340 nm	
	Fresh	Dry
Leaf	2.6	2.7
Flower	2.3	2.4
Stem	1.3	1.1

Repeated each experiment thrice for each sample.

Table 9. Scopoletin identification in water plant extracts of *Wedelia trilobata* by spectrophotometric method

Sample	OD at 340 nm	
	Fresh	Dry
Leaf	3.3	2.8
Flower	3.3	2.3
Stem	1.6	1.6

Repeated each experiment thrice for each sample.

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). From results, we found strong antioxidants, antimicrobial and anti inflammatory activities specifically in the water leaf and stem extracts of *W. trilobata*. High TPC values found in water 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., 2009), antimicrobial (Kaur and Aroara, 2009) and anti inflammatory activity (Sakat et al., 2010). These flavonoids have been found to possess antioxidants, antimicrobial and anti inflammatory properties in various studies (Cushnie and Lamb, 2005).

Strong presence of tannins in all extracts may explain its potent bioactivities as tannins are known to possess

potent antioxidants (Zhang and Lin, 2008), antibacterial activities (Kaur and Arora, 2009) and anti-inflammatory properties (Fawole et al., 2010). Tannins exert the antimicrobial action by precipitating the microbial proteins (Scalbert, 1991). The antimicrobial activity was observed from phenol (Pelczar, 1988), saponins (Soetan et al., 2006) and terpenoids (Singh and Singh, 2003). 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). The presence of terpenoids has shown as antimicrobial (Singh and Singh, 2003), antioxidant (Grassman, 2005) and anti-inflammatory properties (Neichi et al., 1983). Cardiac glycosides are the secondary metabolites in plants, they usually toxic and have drug like therapeutic agents. All extracts exhibited the presence of cardiac glycosides are known to possess potent antimicrobial (De et al., 2009), antioxidant (Ayoola et al., 2008), and anti-inflammatory activities (Kumar and Kumud, 2010). Plant coumarins have been found to be possessing strong antimicrobial (Ojala et al., 2000), antioxidant (Torres et al., 2006) and antinflammatory activity (Garcia-Argez et al., 2000).

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. 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 neutraphils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage (Chou et al., 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.

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 antinflammatory activities of many plants (Tubaro et al., 1988). Hence, the presence of bioactive compounds in the water extract of different parts of *W. trilobata* may contribute to its, antimicrobial, antioxidant and anti inflammatory activity. Coumarin is a chemical which is found naturally in plants. Esculetin and scopoletin are the two different types of the compounds, they also naturally present in many plants. Both coumarins were observed in leaf at higher concentration followed by stem and flower. Esculetin (6,7-dihydroxy-2*H*-1-benzopyran- 2-one) is a coumarin derivative found in various natural plant products and has been reported to have beneficial biological and biochemical activities. For example, esculetin has been shown to have an anti-inflammatory effect in the croton oil ear test (Tubaro et al., 1988), anti-proliferative effects on vascular smooth muscle cells (Huang et al., 1993), and inhibitory action on *N*-methyl-*N*-nitrosourea-induced mammary carcinoma (Hecht et al., 1999); in addition, it is a scavenger of oxygen free radicals (Lin et al., 2000). It has also been shown to have inhibitory effects on 5- and 12-lipoxygenases of cloned mastocytoma cells (Neichi et al., 1983). Esculetin induces apoptosis of human leukemia HL-60 cells (Chu et al., 2001) and suppresses cancer cell proliferation (Hofmanova et al., 1996). Pan et al. (2003) reported that esculetin inhibited Ras-mediated cell proliferation. Scopoletin is exhibited as antioxidant (Shaw et al., 2003) and anti-inflammatory (Moon et al., 2007).

The present investigation has shown that the fresh parts of leaf and stem water extracts of *W. trilobata* have shown the presence of active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The water leaf and stem extract from fresh and dry parts 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 water extract. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols, Saponins and coumarins. 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.

Conclusions

In the present study results indicate that the leaves and flower extracts of *W. trilobata* possess antimicrobial, antioxidant and anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols, saponins and coumarins. The extracts showed strong antibacterial activity compared with the fungi. The extracts serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation and

proteinase activity and stabilized the red blood cells membrane. These reports provide a basic scientific evidence to support its traditional medicinal uses. In this study might suggest a possible use of *W. trilobata* as source of natural antibacterial, antioxidant and anti-inflammatory agent.

ACKNOWLEDGEMENTS

We thank Dr. MR Hulinaykar, Managing Trustee, Sri Shridevi Charitable Trust (R.) and Dr. MA Venkatesh, Principal, SIET, Tumkur, India for their encouragement.

REFERENCES

Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ (2009). Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of *Buddleja saligna*. BMC Complement Altern. Med., 9: 21.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J. Pharmaceut. Res., 7(3): 1019-1024.

Baladrin MF, Klocke JA, Wurtele ES (1985). Natural plant chemicals: sources of industrial and medicinal plants. Sci., 228: 1154-1160.

Bandow JE, Brotz H, Leichert LIO, Labischinski H, Hecker M (2003). Proteomic approach to understanding antibiotic action. Amicro. Agents. Chemother., 47: 948-955.

Berahou AA, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA (2007). Antibacterial activity of *Quercus ilex* bark's extracts. J. Ethnopharmacol., 112: 426-429.

Block LC, Santos AR, de Souza MM, Scheidt C, Yunes RA, Santos MA, Monache FD, Cechinel Filho V (1998). Chemical and pharmacological examination of antinociceptive constituents of *Wedelia paludosa*. J. Ethnopharm., 61(1): 85-89.

Chou CT (1997). The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant induced paw edema in rats and inflammatory mediators release. Phytother. Res., 11:152-154.

Chu CY, Tsai YY, Wang CJ, Lin WL, Tseng TH (2001). Induction of apoptosis by esculetin in human leukemia cells. Eur J. Pharmacol., 416: 25-32.

Cotran RS, Kumar V, Robbins SL (1994). Pathologic basis of disease. Philadelphia. Saunders WB company.

Cushnie TPT, Lamb AJ (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.

Das SN, Chatterjee S (1995). Long term toxicity study of ART-400. Indian Indg. Med., 16(2): 117-123.

De N, Maori L, Ardo H (2009). A study on antimicrobial effect of extracts of *Cassia arereh* (Del.) on some clinical isolates. J. Med. l Plants Res., 3(3): 116-119.

Espin JC, Soler-rivas C, Wichers HJ (2000). Characterization of the total free radical scavenging capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. J. Agric. Food Chem., 48: 648-656.

Fawole OA, Amoo SO, Ndhlala AR, Light ME, Finnie JF, Van Staden J (2010). Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. J. Ethnopharmacol., 127(2): 235-241.

Garcia-Argez AN, Apan RTO, Delago PH, Velazquez G, Martinez-Vazquez M (2000). Anti-inflammatory activity of coumarins from *Decatropis bicolor* on TPA ear mice model. Planta Medica, 66(3): 279-281.

Gepdireman A, Mshvildadze V, Suleyman H and Elias R (2005). Acute anti-inflammatory activity of four saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. Phytomedicine, 12(6-7): 440-444.

Gilham B, Papachristodoulou K, Thomas JH (1997). Wills Biochemical Basis of medicine. Oxford: Butterworth-Heinemenn.

Grassman J (2005). Terpenoids as plant antioxidants. Vitamins and Hormones, 72: 505-535.

Greenwald P, Clifford CK, Miner JA (2001). Diet and cancer prevention. Euro. J. Cancer, 37: 948-965.

Gulcin I, Mshvildadze V, Gepdiremen A, Elias R (2004). Antioxidant activity of saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. Planta Medica, 70(6): 561-563.

Halliwell B (1996). Antioxidants in human health and disease. Annu. Rev. Nutr., 6: 33-50.

Halliwell B, Gutteridge JMC (1989). Free radicals in biology and medicine (2nd ed). Japan Scientific Societies Press, Tokyo, Japan, pp. 229-233.

Hecht SS, Kenney PM, Wang M, Trushin N, Agarwal S, Rao AV, Upadhyaya P (1999). Evaluation of butylated hydroxyanisole, myo-inositol, curcumin, esculetin, resveratrol and lycopene as inhibitors of benzo[a]pyrene plus 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. Cancer Lett., 137: 123-130.

Hofmanova J, Musilova E, Kozubik A (1996). Suppression of human cancer cell proliferation by lipoxygenase inhibitors and gammaradiation *in vitro*. Gen. Physiol. Biophys., 15: 317-331.

Honda K, Casadesus G, Paterson RB, Perry G, Smith MA (2004). Oxidative stress and redox iron in Alzheimer's disease. Ann. New York Acad. Sci., 1012: 179-182.

Huang HC, Lai MW, Wang HR, Chung YL, Hsieh LM, Chen CC (1993). Antiproliferative effect of esculetin on vascular smooth muscle cells: possible roles of signal transduction pathways. Eur. J. Pharmacol., 237: 39-44.

Iwu MW, Duncan AR, Okunji CO (1999). New antimalarials of plant origin. In: Janick J, editor. Perspective on new crops and new uses. Alexandria: VA ASHS Press, pp. 457-462.

Kaur GJ, Arora DS (2009). Antibacterial and Phytochemical screening of *Anthum graveolens, Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complement Altern. Med., 9: 30.

Kumar SM, Kumud U (2010). Anti-inflammatory activity of root of *Dalbergia sissoo* (Rox.b) in Carrageenan-Induced paw edema in rats. Pharmacognosy J., 2(11): 427-430.

Lai HY, Lim YY, Tan SP (2009). Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Biosci. Biotechnol. Biochem., 73: 1362-1366.

Lans C (1996). Ethnoveterinary practices used by livestock keepers in Trinidad and Tobago. In *Unpublished M.Sc. thesis*. Wageningen: Agricultural University, Department Ecological Agriculture, the Netherlands.

Lewis DA (1989). Anti-inflammatory drugs from plants and marine sources. Basel: Bikhauser Verlag, 1989.

Lin WL, Wang CJ, Tsai YY, Liu CL, Hwang JM, Tseng TH (2000). Inhibitory effect of esculetin on oxidative damage induced by t-butyl hydroperoxide in rat liver. Arch. Toxicol., 74: 467-472.

Liyana-Pathirana CM, Shahidi F (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J. Agric. Food Chem., 53: 2433-2440.

Maldini M, Sosa S, Montoro P, Giangaspero A, Balick MJ, Pizza C, Della Loggia R (2009). Screening of the topical anti-inflammatory activity of the bark of *Acacia cornigera* Willdenow, *Byrsonima crassifolia* Kunth, *Sweetia panamensis* Yakovlev and the leaves of *Sphagneticola trilobata* Hitchcock. J. Ethanopharmacol., 122(3): 430-433.

Mandal P, Babu SSP, Mandal NC (2005). Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterepia, 76(5): 462-465.

Miquel J, Romano-Bosca A (2004). Oxidative stress and antioxidant diet supplementation in ageing, arterosclerotic and immune dysfunction processes. ARS Pharm., 45(2): 91-109.

Moon PD, Lee BH, Jeong HJ, An HJ, Park SJ, Kim HR, Ko SG, Um JY, Hong SH, Kim HM (2007). Use of scopoletin to inhibit the production of inflammatory cytokines through inhibition of the IκB/NF-κB signal cascade in the human mast cell line HMC-1. Eur. J. Pharmacol., 555(2-3): 218-225.

Mortino E, Ramaiola I, Urbano M, Bracco F and Collina S. 2006. Microwave assisted extraction of coumarin and related compounds

from *Melilotus officinalis* (L.) Pallas as an alternative to Soxhlet and ultrasound assisted extraction. J. Chromatography A., 1125: 147-151.
Neichi T, Koshihara Y, Murota S (1983). Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. Biochem. Biophys. Acta, 753: 130 -132.
Obdoni BO, Ochuko PO (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extract of some Homostatic Plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci., 8b: 203-208.
Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, Vuorela P (2000). Antimicrobial activity of some coumarin containing herbal plants growing in Finland. J. Ethnopharmacol., 73(1-2): 299-305.
Pan SL, Huang YW, Guh JH, Chang YL, Peng CY and Teng CM (2003). Esculetin inhibits Ras-mediated cell proliferation and attenuates vascular restenosis following angioplasty in rats. Biochem. Pharmacol., 65: 1897-1905.
Pelczar MJ, Chan, ECS, Krieg NR (1988). Control of microorganisms, the control of microorganisms by physical agents. Microbiology, 469-509.
Rodders J, Speedie M, Tyler V (1996). Pharmacognosy and harmacobiotecknology. Baltimore: Williams and Wilkins, pp. 1-14.
Sakat S, Juvekar AR, Gambhire MN (2010). *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int. J. Pharm. Pharmacol. Sci., 2(1): 146-155.
Scalbert A (1991). Antimicrobial properties of tannins. Phytochem, 30: 3875-3883.
Shaw CY, Chen CH, Hsu CC, Chen CC, Tsai YC (2003). Antioxidant properties of scopoletin isolated from *Sinomonium acutum.* Phytother. Res., 17(7): 823-825.
Singh B, Singh S (2003). Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. Phytotherapy Res., 17(7): 814-816
Soetan KO, Oyekunle MA, Aiyelaagbe OO, Fafunso MA (2006). Evaluation of the antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench. Afr. J. Biotechnol., 5 (23): 2405-2407.
Solich P, Polá ek M and Karlí ek R (1995). Sequential flow-injection spectrofluorimetric determination of coumarins using a double-injection single-line system. Analytica Chimica Acta., 308(1-3): 293-298.
Szollosi R, Szollosi Varga I (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). Acta Biologica Szegediensis, 46: 125-127.
Taddei A and Rosas-Romero AJ (1999). Antimicrobial activity of *Wedelia trilobata* crude extracts. Phytomedicine, 6(2): 133-134.
Tamma RV, Miller GC (1985). High performance liquid chromatographic analysis of coumarin and flavonoids from sections of tridentate of *Artemesia*. J. Chromatography, 322: 266-269.
Torres R, Faini F, Modak B, Urbina F, Labbé C, Guerrero J (2006). Antioxidant activity of coumarins and flavonols from the resinous exudate of *Haplopappus multifolius*. Phytochemistry, 67(10): 984-987.
Tubaro A, Del Negro P, Ragazzi E, Zampiron S, Della Loggia R (1988). Anti-inflammatory and peripheral analgesic activity of esculetin *in vivo*. Pharmacol. Res. Commun, 8: 83-85.
Verastegue A, Verde J, Garcia S, Heredia N, Oranday A, Rivas C (2008). Species of *Agave* with antimicrobial activity against selected pathogenic bacteria and fungi. World J. Micribiol. Biotechnol. 24: 1249-1252.
Verastegue MA, Sanchez CA, Heredia NL, Garcia AJS (1996). Antimicrobial activity of extracts of three major plants from the Chihauhuan desert. J. Ethnopharmacol., 52: 175-177.
Yen GC, Duh PD (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. J. Agric. Food Chem., 42: 629-632.
Zhang LL, Lin YM (2008). Tannins from *Canarium album* with potent antioxidant activity. J. Zhejiang Univ. Sci., B, 9: 407-415.