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Full Length Research Paper

Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity and phytochemical screening of *Crotalaria pallida* Aiton

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The antimicrobial, antioxidant and anti-inflammatory activities, lipoxygenase, xanthine oxidase (XO), acetylcholinesterase activities and phenolic contents of different solvent extracts (ethanol, ethyl acetate, chloroform, petroleum ether and water) of *Crotalaria pallida* were evaluated using *in vitro* standard methods. These solvent extracts were most potent inhibiting all isolates with different zones of inhibition. The maximum inhibition of bacteria and fungi was observed from ethanol extract. The minimum microbial concentration (MMC) of the active extract was observed from ethanol, petroleum ether and ethyl acetate ranged from 0.3 to 3.2 mg/ml for the sensitive bacteria. In case of fungi, the minimum inhibitory concentration (MIC) of the active extracts ranged from 0.6 to 4.0 mg/ml. These data suggest that the *C. pallida* extracts analyzed are potential antimicrobial candidates with a broad range of activity. Phytochemical analysis was conducted to all the solvent extracts to their constituents. The level of total phenol, alkaloids, terpenoids, saponins, phenols, steroids and tannins from ethanol, ethyl acetate and petroleum ether extracts were higher. The antioxidant activities of different solvent extracts of *C. pallida* were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferrous reducing antioxidant property (FRAP) methods. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standard for antioxidant activity. The ethanol, ethyl acetate and petroleum ether extracts possessed strong scavenging activity in both DPPH and FRAP methods. The ethanol, ethyl acetate and petroleum ether had showed free radical inhibition of 88, 72 and 73 and 3617.89 ± 0.03, 2189.33 ± 0.03 and 1133.26 ± 0.01, respectively. The *in vitro* anti-inflammatory activities were evaluated using albumin denaturation, membrane stabilization and proteinase inhibitory activities using all the solvent extracts. The ethanol, ethyl acetate and petroleum ether showed activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization with 83.17, 71.33 and 58.14 and 68.21, 61.44 and 60.72 g/ml, respectively. The proteinase activity was significantly inhibited by the ethanol (82.53), ethyl acetate 74.31) and petroleum ether (62.92) g/ml. Aspirin was used as standard drug for the study of anti-inflammatory activity. In addition, the ethanol, ethyl acetate and petroleum ether extracts showed anti-lipoxygenase activity and they also exhibited a moderate xanthine oxidase and acetylcholinesterase inhibitory activity.

Key words: *Crotalaria pallida*, antioxidant, anti-inflammatory, antimicrobial, lipoxygenase, xanthine oxidase.

INTRODUCTION

Due to the 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 micro-organisms. Numbers of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes (Berahou et al., 2007). Natural products derived from plants offer a new

source of biological activities that make a great impact on infectious disease and overall human health (Conner, 1993; Balandrin et al., 1985). Phytochemicals from medicinal plants showing antimicrobial activities have the potential bioactive compounds, because their structures are different from those of more studied microbial sources and therefore their mode of action may also differ, very likely (Fabricant and Fansworth, 2001). There is a growing interest in correlating the phytochemical constituents of a medicinal plant with pharmacological activity (Prachayasittikul et al., 2008; Costa et al., 2008). Screening active compounds from plants have lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Kumar et al., 2004; Sheeja and Kuttan, 2007; Mukherjee et al., 2007).

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids and DNA, and initiate degenerative diseases. Antioxidants compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals, such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The preservative effective of many plant species and herbs suggests the presence of antioxidant and antimicrobial constituents in their tissues. Many medicinal plants contain large amounts of antioxidants (Javanmardi et al., 2003; Prakash, 2001).

Antioxidants are compounds that inhibit or setback the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing bio molecules, resulting in cell death or tissue damage. Oxidative damage plays a significantly pathological role in human disease. Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions, including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases, liver diseases, cancer and AIDS (Halliwell and Gutteridge, 1984; Pourmorad et al., 2006; Halliwell and Gutteridge, 1989). Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Dasgupta, 2004; David et al., 2004). The antioxidants can neutralize the sick effects of free radicals by scavenging or chain breaking (like vitamin A, C, β-carotene, etc.) or some other mechanism of action. These antioxidants must be constantly replenished since they are 'used up' in the process of neutralizing free radicals (Kumar and Sharma, 2006). 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 ROS such as superoxide anions, hydroxyl radicals and H_2O_2 are generated (Aruoma, 1999). ROS are involved in an organism's vital activities, including phagocytosis, regulation of cell proliferation, intracellular signaling and synthesis of biologically active compounds (Halliwell and Gutteridge, 1989; Miquel and Romano-Bosca, 2004).

Inflammation is the reaction of living tissues to injury, infection or irritation. It involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane and Botting, 1995) which are aimed at host defense and usually activated in most disease condition. In many inflammatory disorders, there is excessive activation of phagocytes, production of O_2-, OH radicals as well as non free radicals species (H_2O_2) (Gilham et al., 1997), which can harm tissues severely 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 the 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 have been treated by the administration of plant extracts from medicinal plants (Borek, 1997), the present investigation was aimed at evaluating the antimicrobial, antioxidant and anti-inflammatory potential of different solvent extract of *Crotalaria pallida.* The literature survey indicates that no reports are available from India regarding antimicrobial, antioxidant and anti-inflammatory activity of *C. pallida*. 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,

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India.

Extract preparation

Whole plant was air dried at room temperature for 4 weeks to get consistent weight. The dried parts were later ground to powder. 100 g of wet and dried samples were extracted with distilled water, ethyl acetate, ethanol, chloroform and petroleum ether (60 to 80°C, 200 ml for dry parts) for 2 days in water both with a shaking attachment separately for each solvent. Each extracts were lyophilized under 5 µm mercury (Hg) pressure and were stored at -20°C separately. The experiment was carried out using an appropriate amount of lyophilized material.

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, terpenoids, steroids, phenol, alkaloids (Obdoni and Ochuko, 2001) and tannins (Kaur and Arora, 2009), and 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 each solvent extracts 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/g plant extract. All tests were performed in triplicate.

Determination of antimicrobial activity

Antimicrobial assay

Pseudomonas fluorescens, *Clavibacter michiganensis* sub species *michiganensis*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum* and strains of *Staphylococcus aureus, Escherichia 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 *C. michiganensis* sub spp. *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 nidulans, Aspergillus flaviceps, Alternaria carthami, Alternaria helianthi, Cercospora carthami, Fusarium solani, Fusarium oxysporum, Fusarium verticilloides* and *Nigrospora oryzae* were obtained from the 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) and Adedapo et al. (2008). 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 media, respectively. Each solvent extracts were dissolved in respective solvents to a 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 chloramphenicol (10 µg) and carbendazim 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 Rotimi et al. (1988) and 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 different solvent extracts of *C. pallida* added in 0.1 mg/ml increments separately. Cultures were incubated at 37°C for 24 h 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 potato dextrose agar (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 been divided into thirds and each section was streaked uniformly with 1×10^3 fungi spores or infective mycelia particles. Fungi were incubated for 10 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 10× 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.

Ferrous reducing antioxidant property (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 µl) (0.5 mg/ml) dissolved in water was added in 4.5 ml of freshly prepared FRAP reagent and was 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 000 µmol/L) was used and results were expressed in µmol 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 dissolving 24 mg DPPH in 100 ml water, stored at -20°C before use. 150 µl of sample (1 0 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 were expressed in µm AA/g fresh mass. Additional dilution is needed if the DPPH value measured will be more than 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 (Katalinic et al., 2006). All determinations were performed in triplicate for all experiments. The percentage of inhibition of DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994):

Percentage of inhibition (%) = [{$Abs_{control}$ - Abs_{sample}}/$Abs_{control}$] × 100

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

Methods of Mizushima and Kobayashi (1968) and Sakat et al. (2010) were followed with minor modifications. The reaction mixture 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 i ncubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percentage of inhibition of protein denaturation was calculated as follows:

Percentage of inhibition (%) = [{$Abs_{control}$- Abs_{sample}}/$Abs_{control}$] × 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 (Sadique et al., 1989; Saket et al., 2010).

Heat induced hemolytic: The reaction mixture (2 ml) consists 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 solvent extract samples. Percentage of membrane stabilization activity was calculated by the formula mentioned earlier (Shinde et al., 1999; Saket et al., 2010).

Protein inhibitory action: The test was performed according to the modified method of Oyedepo and Femurewa (1995) and Sakat et al. (2010). The reaction mixture (2 ml) 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 reaction mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein wa s 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.

Anti-lipoxygenase activity: Anti-lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme (Shinde et al., 1999). Test solution was dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and was added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) and was incubated for 5 min at 25°C. Af ter which, 1.0 ml of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percentage of inhibition was calculated from the following equation:

Percentage of inhibition (%) = [{$Abs_{control}$- Abs_{sample}}/$Abs_{control}$] × 100

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Xanthine oxidase assay: Xanthine oxidase (XO) activity was assayed spectrophotometrically at 300 nm as described by Yamamoto et al. (1993). Briefly, the reaction mixture consisting of 500 µl of solution A (0.1 M phosphate buffer containing 0.4 mM xanthine and 0.24 mM nitro blue tetrazolium chloride (NBT)), 500 µl of solution B (0.1 M phosphate buffer containing 0.0449 units/ml xanthine oxidase) and 50 µl of a 10% of each solvent extracts were incubated in a cuvette at 37°C for 20 min. The enzym e activity was expressed as the increment in absorption at 300 nm per unit time.

Acetylcholinesterase (AChE) inhibitory activity: The AChE inhibitory assay and inhibition kinetics analysis were conducted according to the protocol described by Lopez et al. (2002) with some modifications. The assay mixture consist of 200 µl of Tris-HCl 50 mM, pH 8.0, 0.1% BSA buffer, 100 µl of extracts or fractions solution (final concentration: 100 µg/ml) was dissolved in buffer-MeOH (10%) and 100 µl of AChE (0.22 U/ml). The mixture was incubated at room temperature for 2 min before the addition of 500 µl of DTNB (5,5 V-dithiobis [2-nitrobenzoic acid]) (3 mM) and 100 µl of substrate acetylthiocholine iodide (ATCI) (15 mM). The developing yellow color was measured at 405 nm after 4 min. Galantamine was used as positive control at a final concentration of 0.2 µg/ml in the assay mixture. AChE inhibitory activity was expressed as percentage of inhibition of AChE, calculated as (1-B/A) × 100, where A is the change in absorbance of the assay without the plant extract (abs. with enzyme and abs. without

Table 1. Zone of inhibition (in mm) of antimicrobial activity by disc diffusion method using different solvent extract of *C. pallida.*

Microorganism	Extract samples with inhibition zone (mm)					
	Ethyl acetate	Ethanol	Petroleum ether	Chloroform	Water	Chloramphenicol
Bacterial pathogens						
K. pneumonia	4 ± 1^{d}	9 ± 1^{d}	7 ± 1^{bc}	2 ± 1^{b}	7 ± 1^{a}	18 ± 2^{cd}
E. coli	4 ± 1^{d}	14 ± 2^{b}	NI	2 ± 1^{b}	2 ± 1^{d}	20 ± 2^{b}
S. aureus	6 ± 1^{c}	9 ± 1^{d}	6 ± 1^{c}	2 ± 1^{b}	2 ± 1^{d}	18 ± 2^{cd}
P. aeruginosa	6 ± 1^{c}	8 ± 1^{de}	6 ± 1^{c}	4 ± 1^{b}	6 ± 1^{ab}	18 ± 2^{cd}
P. fluorescens	3 ± 1^{de}	8 ± 1^{de}	2 ± 1^{e}	2 ± 1^{b}	4 ± 1^{c}	21 ± 2^{ab}
C. michiganensis sub spp. *michiganensis*	8 ± 1^{b}	13 ± 2^{bc}	8 ± 1^{b}	2 ± 1^{b}	6 ± 1^{ab}	16 ± 2^{d}
X. oryzae pv. *oryzae*	4 ± 1^{d}	6 ± 1^{f}	4 ± 1^{d}	2 ± 1^{b}	2 ± 1^{d}	16 ± 2^{d}
X. axanopodis pv. *malvacearum*	12 ± 2^{a}	16 ± 2^{a}	14 ± 2^{a}	2 ± 1^{b}	6 ± 1^{ab}	22 ± 2^{a}
Fungal pathogens						**Carbendazim**
A. nidulans	6 ± 1^{c}	14 ± 2b	6 ± 1^{c}	2 ± 1^{b}	5 ± 1^{b}	21 ± 2^{ab}
A. flaviceps	6 ± 1^{c}	11 ± 1^{cd}	4 ± 1^{d}	2 ± 1^{b}	4 ± 1^{c}	21 ± 2^{ab}
A. carthami	5 ± 1^{cd}	8 ± 1^{de}	4 ± 1^{d}	2 ± 1^{b}	4 ± 1^{c}	19 ± 2^{c}
A. helianthi	4 ± 1^{d}	6 ± 1^{f}	4 ± 1^{d}	2 ± 1^{b}	4 ± 1^{c}	21 ± 2^{ab}
C. carthami	2 ± 1^{e}	6 ± 1^{f}	4 ± 1^{d}	2 ± 1^{b}	4 ± 1^{c}	21 ± 2^{ab}
F. solani	2 ± 1^{e}	2 ± 1^{g}	2 ± 1^{e}	2 ± 1^{b}	2 ± 1^{d}	19 ± 2^{c}
F. oxysporum	2 ± 1^{e}	2 ± 1^{g}	2 ± 1^{e}	2 ± 1^{b}	2 ± 1^{d}	19 ± 2^{c}
F. verticilloides	2 ± 1^{e}	2 ± 1^{g}	2 ± 1^{e}	2 ± 1^{b}	2 ± 1^{d}	20 ± 2^{b}
N. oryzae	4 ± 1^{d}	3 ± 1^{g}	4 ± 1^{d}	2 ± 1^{b}	2 ± 1^{d}	20 ± 2^{b}

+: Presence; -: absence, repeated the each experiments three times for each replicates. NI-No Inhibition

enzyme), and B is the change in absorbance of the assay with the plant extract (abs. with enzyme and abs. without enzyme).

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

Antimicrobial assay

The antimicrobial activities of the different solvent extracts of *C. pallida* are presented in Table1. The ethanol extract of *C. pallida* is much higher than other solvent extracts; the order in decree-sing antibacterial activity was petroleum ether, ethyl acetate, water and chloroform.

The highest activity of ethanol extract observed on *X. axanopodis* pv. *malvacearum*, *E. coli* and *C. michiganensis* sub spp. *michiganensis*. In anti-fungal activity, the ethanol extract showed more activity followed by petroleum ether, ethyl acetate, water and chloroform. All the solvent extracts exhibited higher activity on all species of bacteria

Table 2. Minimal antimicrobial activity of different extract of *C. pallida.*

Sample	Ec	Pa	Sa	Kp	Pf	Cmm	Xoo	Xam
Ethyl acetate	2.0 ± 0.4^{a}	2.0+0.4^{a}	2.0 + 0.3^{a}	2.0 + 0.3^{b}	1.3 + 0.4^{b}	3.8 + 0.4^{a}	1.4 + 0.05^{b}	2.4 + 0.5^{c}
Ethanol	1.8 ± 0.3^{b}	1.5 ± 0.3^{b}	1.8 + 0.4^{b}	1.8 + 0.4^{c}	1.9 + 0.06^{a}	3.5 + 0.3^{b}	2.3 + 0.13^{a}	3.2 + 0.5^{a}
Petroleum ether	1.4 ± 0.5^{c}	2.0 ± 0.3^{a}	2.0 + 0.3^{a}	3.4 + 0.5^{a}	1.3 + 0.04^{b}	3.5 + 0.3^{b}	1.4 + 0.05^{b}	2.8 + 0.5^{b}
Chloroform	0.3 ± 0.3^{d}	2.0 ± 0.3^{a}	2.0 + 0.3^{a}	2.0 + 0.3^{b}	0.3 + 0.03^{d}	2.0 + 0.3^{c}	0.3 + 0.03^{d}	1.3 + 0.03^{e}
Water extract	1.4 ± 0.5^{c}	2.0 ± 0.3^{a}	2.0 + 0.3^{a}	3.4 + 0.3^{a}	1.1 + 0.04^{c}	1.2 + 0.4^{d}	0.9 + 0.07^{c}	1.6 + 0.3^{d}
Chloromphenicol	0.02 ± 0.02^{e}	0.02 ± 0.02^{c}	0.02 ± 0.02^{c}	0.04 ± 0.02^{d}	0.02 ± 0.02^{e}	0.02 ± 0.02^{e}	0.02 ± 0.02^{e}	0.02 ± 0.02^{f}

NI, No inhibition, Ec, *Escherichia. Col;,* Pa*, Pseudomonas aeruginosa*; Sa,*Staphylococcus aureus*; Kp,*Klebsiella pneumonia*; Pf,*Pseudomonas fluorescens*; Cmm, *Clavibacter michiganensis* sub spp. *Michiganensis*; Xoo, *Xanthomonas oryzae* pv. *oryzae*, and Xam, *Xanthomonas axanopodis* pv. *Malvacearum*. 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.

Table 3. Minimal antimicrobial activity of different extract of *C. pallida*.

Extract	Anid	Afla	Ac	Ah	Cc	Fs	Fo	Fv	No
Ethyl acetate	1.0 + 0.3^{b}	1.0 + 0.3^{b}	1.2 + 0.3^{c}	0.9 + 0.3^{b}	0.6 + 0.3^{c}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	1.2 + 0.3^{a}
Ethanol	4.0 + 0.3^{a}	3.0 + 0.3^{a}	3.2 + 0.3^{a}	1.0 + 0.3^{a}	1.8 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.9 + 0.3^{b}
Petroleum ether	1.0 + 0.3^{b}	1.0 + 0.3^{b}	1.9 + 0.3^{b}	0.5 + 0.3^{d}	1.2 + 0.3^{b}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	1.2 + 0.3^{a}
Chloroform	0.8 + 0.3^{d}	0.9 + 0.3^{c}	0.8 + 0.3^{d}	0.9 + 0.3^{b}	0.6 + 0.3^{c}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{c}
Water	0.9 + 0.3^{c}	0.8 + 0.3^{d}	0.8 + 0.3^{d}	0.8 + 0.3^{c}	1.2 + 0.3^{b}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{a}	0.6 + 0.3^{c}
Carbendazim	0.03 ± 0.03^{f}	0.03 ± 0.3^{e}	0.05 ± 0.04^{e}	0.03 ± 0.03^{e}	0.05 ± 0.04^{d}	0.03 ± 0.03^{b}	0.03 ± 0.03^{b}	0.03 ± 0.03^{b}	0.05 ± 0.0^{d}

NI, No inhibition, 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*. 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.

and fungi. The *Aspergillus* species were inhibited by ethanol extract strongly followed by *Alternaria* species, *Cercospora carthami*, *Fusarium* species and *N. oryzae*. Other extracts also inhibited all fungi moderately. Almost all the solvent extracts had inhibited the growth of tested micro-organisms, whereas, standard chloramphenicol for bacteria and carbendazim for fungi have shown significant inhibition on all bacteria and fungi, respectively. The MMC and MIC of different solvent extract of *C. pallida* against bacteria ranged from 0.3 to 3.2 mg/ml (Table 2). The ethanol extract was more active against all bacterial strains. The MMC for bacteria and the MIC for fungi of *C. pallida* wet parts extracts ranged from 0.6 to 4.0 g/ml for all the organisms tested (Table 3). The different solvent extracts analyzed here are potential candidates for broadly active antimicrobial compounds. Although, the antimicrobial activity of the whole plant extracts are low as compared to purified antibiotics or fungicide (Tables 2 and 3), purification of the active natural compounds could derive a metabolite more active.

Table 4. Phytochemical analysis for the different solvent extracts of *C. pallida*.

Extract	Alkaloids	Flavonoids	Terpenoids	Saponins	Phenols	Steroids	Tannins
Petroleum ether	++	++	+	+	++	+	+
Ethanol	+++	+++	+++	+++	+++	+++	+++
Ethyl acetate	++	++	++	++	++	++	++
Chloroform	-	-	-	-	-	-	-
Water	+	+	+	+	+	+	+

+++, Strong; ++, medium; +, poor presence; -: absence; the experiments was repeated three times for each replicates. Classification was based on observation of colour intensity and amount of precipitate. 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 5. Total phenolic content and total antioxidant activity from different solvent extract of *C. pallida*.

Extract	FRAP (µmol/L)	TPC (mg gallic acid/g plant material)
Ethyl acetate	1133.26 ± 0.01^{e}	288.17 ± 17^{c}
Ethanol	3617.89 ± 0.03^{a}	310.21 ± 21^{a}
Petroleum ether	2189.33 ± 0.03^{b}	291.44 ± 17^{b}
Chloroform	1005.04 ± 0.03^{f}	254.32 ± 14^{e}
Water	1323.08 ± 0.03^{d}	266.54 ± 16^{d}
Ascorbic acid	1648.52 ± 0.03^{c}	-
BHT	64.84 ± 0.03^{g}	-

All the data is three replicates of each samples. Phenolic antioxidant coefficient calculated as the ratio FRAPS (µM/L). 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.

Phytochemical analysis

The phytochemical screening showed that the different solvent extracts of *C. pallida*, the alkaloids, flavonoids, terpenoids, saponins, phenols, steroids and tannins were present in all the solvent extract except chloroform. The ethanol extract yielded strongly, all the phytochemicals followed by petroleum ether and ethyl acetate. Chloroform extract did not yield any phytochemicals. The water extract also yielded all the phytochemicals at poor presence (Table 4).

Total phenol contents and antioxidant activity

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent and was expressed in terms of mg gallic acid equivalent (GAE)/g plant extract. More TPC was observed in ethanol extract (10.21), followed by petroleum ether (9.44), ethyl acetate (8.17), water (6.54) and chloroform (5.32) (Table 5). The ethanol, petroleum ether and ethyl acetate plant extracts had showed highest TPC.

Total antioxidant power (FRAP)

The reducing ability of the extracts was in the range of 100.04 to 3617.89 µg Fe (II)/g (Table 5). The FRAP values for the extracts were significantly lower than that of ascorbic acid except ethanol and petroleum ether extract but higher than that of butylated hydroxytoluene (BHT).

DPPH radical scavenging activity

Figure 1 shows the dose response curve of DPPH radical scavenging activity of different solvent extracts of *C. pallida* when compared with BHT and ascorbic acid. It was observed that ethanol extract of *C. pallida* had higher activity than that of other solvent extracts. At a concentration of 0.1 mg/ml, the scavenging activity of ethanol extract of *C. pallida* reached above 80% while petroleum ether, ethyl acetate, chloroform and water extracts also reached 60%.

Anti inflammatory properties

Inhibition of albumin denaturation

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mecha-

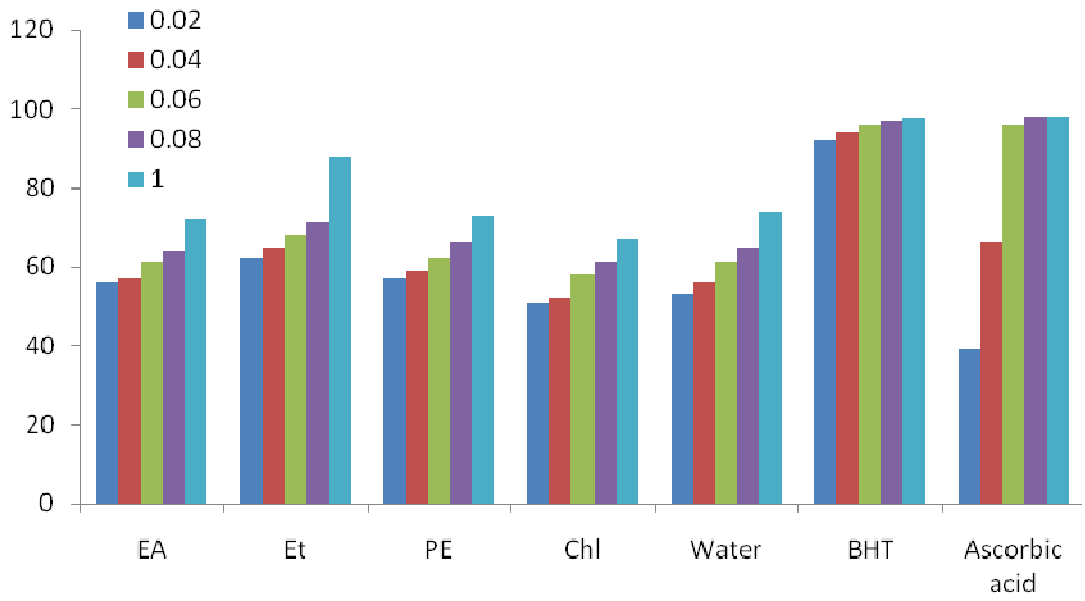

Figure 1. DPPH scavenging activities of the different solvent extracts of *C. pallida.* EA, Ethyl acetate; Et, ethanol; PE, petroleum ether; Chl, chloroform, butylated hydroxytoluene.

Table 6. Effect of different solvent extracts of *C. pallida* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition.

Test sample	Albumin denaturation	Membrane stabilization	Proteinase inhibition
Ethyl acetate	58.14 ± 0.07^{e}	60.72 ± 0.06^{c}	62.92 ± 0.06^{d}
Ethanol	83.17 ± 0.09^{a}	68.21 ± 0.07^{b}	82.53 ± 0.08^{b}
Petroleum ether	71.33 ± 0.08^{c}	61.44 ± 0.05^{c}	74.31 ± 0.07c
Chloroform	52.81 ± 0.06^{f}	51.17 ± 0.04^{d}	57.52 ± 0.05^{f}
Water	59.43 ± 0.06^{d}	52.64 ± 0.04^{d}	59.87 ± 0.05^{e}
Aspirin (200 µg/ml)	75.89 ± 0.06^{b}	85.92 ± 0.02^{a}	92.83 ± 0.03^{a}

Experiments were repeated three times for each replicates. 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.

nism of the anti-inflammation activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table 6). Maximum inhibition 83.17 ± 0.06 was observed from ethanol extract followed by petroleum ether (71.33 ± 0.06), ethyl acetate (58.14 ± 0.07), water (59.43 ± 0.06) and chloroform (52.81 ± 0.06). In all the solvent extracts albumin denaturation was also inhibited, here also, the ethanol extract stood first, followed by petroleum ether and ethyl acetate extracts. 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 to further establish the mechanism of anti-inflammatory action of different water extracts of *C. pallida*. All the solvent 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 6). The maximum inhibition was recorded 68.21 ± 0.06 from ethanol extract followed by petroleum ether (61.44 ± 0.05), ethyl acetate (60.72 ± 0.06), water (52.64 ± 0.05) and chloroform (51.17 ± 0.06). Ethanol extract has shown highest membrane stabilization activity followed by petroleum ether and ethyl acetate. The aspirin standard drug showed the maximum inhibition 85.92%.

Table 7. Inhibition of Xanthine oxidase, acetyl cholinesterase and tyrosinase activities from different extracts of *C. pallida.*

Sample	Inhibitors activities (%)	
	Xanthine oxidase (IC_{50} µg/ml)	Acetyl cholinesterase
Ethyl acetate	33.1 ± 0.94^{c}	6.22 ± 1.14^{d}
Ethanol	39.6 ± 1.13$_{a}$	14.46 ± 1.35^{b}
Petroleum ether	36.3 ± 1.01$_{b}$	8.17 ± 1.32^{c}
Chloroform	33.6 ± 0.94^{c}	5.74 ± 0.97^{e}
Water	33.7 ± 0.94^{c}	5.93 ± 1.08^{e}
Galanthamine (20 µg/ml)	-	50.00 ± 1.36^{a}

Experiments were repeated 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.

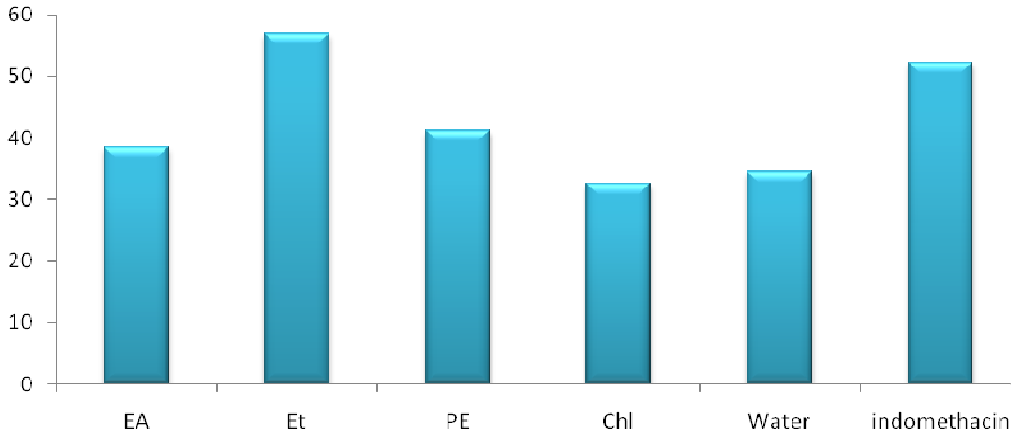

Figure 2. Anti-lipoxygenase activity of different extracts of *C. pallida*. EA: ethyl acetate; Et: ethanol; PE: petroleum ether; Chl: chloroform.

Proteinase inhibitory activity

The *C. pallida* solvent extracts exhibited significant antiproteinase activity. The maximum inhibition was observed from ethanol extract (82.53 ± 0.03) in decreasing order was petroleum ether (74.31 ± 0.03), ethyl acetate (62.92 ± 0.03), water (59.87 ± 0.03) and chloroform (57.52 ± 0.06). The ethanol extract have showed highest proteinase inhibitory activity as compared to petroleum ether and ethyl acetate. The standard drug aspirin have shown that the maximum proteinase inhibitor activity is 92.83 ± 0.03 (Table 7).

Xanthine oxidase assay

The maximum inhibition of xanthine oxidase was observed from ethanol extract (39.6) followed by petroleum ether (36.3), ethyl acetate (34.1), water (33.7) and chloroform (33.6) extracts (Table 7). Maximum inhibition was noticed in ethanol extract as compared to all the solvent extract.

Effect of different solvent extracts on anti-lipoxygenase activity

All the extracts significantly inhibited the lipoxygenase activity; it ranged from 32.43 to 56.88. Ethanol extract showed highest anti-lipoxygenase activity as 56.88 followed by petroleum ether, ethyl acetate, water and chloroform (Figure 2). The standard indomethacin showed a 52.20% inhibition at a concentration of 60 µg/ml.

Acetylcholinesterase inhibitory activity is the first number of requirements for the developments of medicines for treating some diseases. All the solvent extracts were tried for their *in vitro* AChE inhibitory activity at a

concentration of 100 µg/ml and in the assay mixture galanthamine was used as a positive control. Among solvent extracts, the ethanol (14.46 ± 1.35) and petroleum ether (8.17 ± 1.32) extracts exhibited the best AChE inhibitory activity, but these values are lowest to galanthamine inhibitory activity (50%) at 0.2 µg/ml. The other solvent extracts showed moderate effect on inhibition of AChE, and the order of decrease was ethyl acetate (6.22 ± 1.14), water (5.74 ± 1.08) and chloroform (5.74 ± 0.97) (Table 7).

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 the 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 *C. pallida* as a traditional medicine. We found strong antioxidants, antimicrobial and anti-inflammatory activities specifically in the water leaf and stem extracts of *C. pallida*. High TPC values that were found in water leaf and stem extracts (9.41 and 6.81 mg GAE/100 ml) imply the role of phenolic compounds in contributing to these activities. Plant phenolic compounds have been found to possess potent antioxidants (Adedapo et al., 2009; Adesegun et al., 2009; Lai et al., 2010), antimicrobial (Kaur and Arora, 2009; Lai et al., 2010) and anti-inflammatory activity (Sakat et al., 2010; Roy et al., 2010; Garg et al., 2010).

These flavonoids 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).

Strong presence of tannins in all the extracts may explain its potent bioactivities as tannins are known to possess potent antioxidants (Zhang and Lin, 2008), antibacterial activities (Kaur and Arora, 2009; Scalbert, 1991) and anti-inflammatory properties (Souza et al., 2007; Fawole et al., 2010). Tannins exert the antimicrobial action by precipitating the microbial proteins (Scalbert, 1991). The antimicrobial activity was observed from phenol (Pelczar et al., 1988), saponins (Soetan et al., 2006) and terpenoids (Singh and Singh, 2003). Saponins have shown antimicrobial activity (Mandal et al., 2005), antioxidant activity (Gulcin et al., 2004) and anti-inflammatory activity (Gepdireman et al., 2005). The presence of terpenoids have shown antimicrobial (Singh and Singh, 2003), antioxidant (Grassman, 2005) and anti-inflammatory properties (Neichi et al., 1983). Alkaloids are the secondary metabolites in plants, they are usually toxic to various organisms and have drug like therapeutic agents. All extracts that exhibited the presence of alkaloids are known to possess potent antimicrobial (Erdemoglu et al., 2007), antioxidant (Maiza-Benabdesselam et al., 2007) and anti-inflammatory activities (Barbosa-Filho et al., 2006). Plant terpenoids have been found to possess strong antimicrobial (Tincusi et al., 2002), antioxidant (Grassmann, 2005) and anti-inflammatory activities (Neukirch et al., 2005). The plant steroids also showed antimicrobial (Parekh and Chanda, 2007), antioxidant (Nogala-Kalucka et al., 2010) and anti-inflammatory activity (Das et al., 2010).

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 *C. pallida* 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 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 (Luo et al., 2002; Okoli and Akah, 2004). Hence, the presence of bioactive compounds in the water extract of different parts of *C. pallida* may contribute to its, antimicrobial, antioxidant and anti-inflammatory activity.

The present investigation has shown that the different solvent extracts of *C. pallida* have shown the presence of active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The ethanol, ethyl acetate and petroleum ether extracts 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, petroleum ether and ethyl acetate extract fractions. These activities may be due to strong occurrence of polyphenolic compounds, such as alkaloids, flavonoids, tannins, steroids, 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 medicine use and indicate a promising potential for the development of an antimicrobial, antioxidant and anti-inflammatory agent from *C. pallida* 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.

Lipoxygenases (LOXs) are sensitive to antioxidants, and most of their action may consist inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy-radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. The results obtained from our studies on *C. pallida* have shown potential anti-inflammatory activity. The *C. pallida* extracts inhibited the lipoxygenase enzyme activity. This indicates that plant *C. pallida* is more useful in studies of inflammation and in various related physiological studies and diseases, such as aging, cancer, etc.

Conclusions

The present study results indicate that the different solvent extracts of *C. pallida* 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, and saponins. The extracts fractions showed strong antimicrobial activity. The extract fractions serve as free radical inhibitors or scavengers, or they act possibly as primary oxidants. They inhibit the heat induced albumin denaturation and proteinase activity and stabilize the Red Blood Cells membrane. The solvent fractions exhibited a moderate XO inhibitory activity and therefore may be due to the presence of bioactive constituents and these can be useful in the treatment of XO induced diseases. The acetylcholinesterase inhibitory activity was noticed from aforementioned solvent extracts, these can be use for the neurological diseases. Findings from the present study shows that, ethanol, ethyl acetate and petroleum ether extracts attributed potent inhibition of bacteria and fungi and they yielded strong presence of phytochemicals. These extracts also exhibited antioxidant and anti-inflammatory properties strongly. These extracts reduced the activity of lipoxygenase, xanthine oxidase and acetylcholinesterase activities. This report proposing its potential application as a lead compounds for designing potent antimicrobial, antioxidant and anti-inflammatory activity, and they can be used for treatment of various diseases (cancer, neurological disorder, aging and inflammatory).

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REFERENCES

Adedapo AA, Jimoh FO, Koduru S, Anthony Afolayan AJ, Masika PJ (2008). Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea.* BMC Complement Altern. Med., 8: 53.

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.

Adesegun SA, Fajana A, Orabueze CI, Coker HAB (2009). Evaluation of antioxidant properties of *Phaulopsis fascisepala* C.B.Cl. (Acanthaceae). Evid Based Complement Altern. Med., pp. 227-231.

Amarlal S, Mira L, Nogueira JM, Da Silva AP, Florencio MH (2009). Plant extracts with anti-inflammatory properties--a new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. Bioorg. Med. Chem., 17(5): 1876-1883.

Aruoma OI (1999). Free radicals, oxidative stress, and antioxidants in human health and disease. J. Agric. Food Chem, 47: 397-492.

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

Barbosa-Filho JM, Piuvezam MR, Moura MD, Silva MS, Lima KVB, Leitão da-Cunha EV, Fechine IM, Takemura OS (2006). Anti-inflammatory activity of alkaloids: a twenty-century review. Revista Brasileira de Farmacognosia, 16(1): 109-139.

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.

Borek B (1997). Antioxidants and cancer. Sci. Med. (Phila), 4: 51-62.

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.

Conner DE (1993). Naturally occurring compounds. In: Davidson PM, Branen AL (eds) Antimicrobials in foods, 2nd edn. Marcel Dekker Inc., New York.

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

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

Das S, Haldar PK, Pramanik G, Suresh RB (2010). Evaluation of anti-Inflammatory activity of *Clerodendron infortunatum* Linn. extract in rats. Global J. Pharmacol., 4 (1): 48-50.

Dasgupta N, De B (2004). Antioxidant activity of Piper beetle leaf extract *in vitro*. *Food Chem.,* 88: 219-224.

David JM, Barreisors AIBS, David JP (2004). Antioxidant phenyl propanoid esters of triterpenes from *Dioclea tasiophyllla.* Pharm. Biol., 42: 36-38.

Erdemoglu N, Ozkan S, Tosun F (2007). Alkaloid profile and antimicrobial activity of *Lupinus angustifolius* L. alkaloid extract. Phytochem. Rev., 6(1): 197-201.

Fabricant DS, Fansworth NR (2001). The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect., 109: 69-75.

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. Ethnopahmacol., 127(2): 235-241.
Garg VKR, Jain M, Sharma PKR, Garg G (2010). Anti inflammatory activity of *Spinacia oleracea*, Int. J. Pharm. Prof. Res., 1(1): 1-4.
Gepdireman A, Mshvildadze V, Suleyman H, 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 Hormones, 72: 505-535.
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 J (1984). Oxygen toxicity oxygen radicals transition metals and diseases. Biochem. J., 219: 1-4.
Halliwell B, Gutteridge J (1989). Free radicals in biology and medicine. Tokyo; Japan Scientific Press.
Javanmardi J, Stushnoff C, Lockeb E, Vivanco JM (2003). Antioxidant activity and total henolic content of Iranian *Ocimum* accessions. Food Chem., 83: 547-550.
Katalinic V, Milos M, Kusilic T, Jukic M (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem., 94: 550-557.
Kaur GJ, Aroara DS (2009). Antibacterial and Phytochemical screening of Anthum graveolens, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complement Altern., 9: 30.
Kumar N, Sharma SK (2006). Antioxidant studies on some plants; a review. *Hamdard Medicus,* 4: 25-36.
Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S (2004). Anticancer and immunostimulatory compounds from *Andrographis paniculata*, J. Ethnopharm., 92: 291-295.
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.
Lai HY, Yau YY, Kim KH (2010). *Blechnum orientale* Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. BMC Complement Altern. Med., 10: 15.
Lewis DA (1989). Anti-inflammatory drugs from plants and marine sources. Basel: Bikhauser Verlag.
Lin Y, Shi R, Wang X, Shen HM (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. Curr. Cancer Drug Targets, 8: 634-46.
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., 32: 2433–2440.
Lopez S, Bstida J, Viladomat F, Codina C (2002). Acetylcholinesterase inhibitory activity of some amaryllidaseae alkaloids and Narcissus extracts. Life Sci., 71: 2521-2529.
Lopez-Lazaro M (2009). Distribution and biological activities of the flavonoid luteolin, Mini Rev. Med. Chem., 9: 1-59.
Luo XD, Basile MJ, Kennelly EJ (2002). Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (star apple). J. Agric. Food Chem, 50: 1379-1382.
Maiza-Benabdesselam F, Khentache S, Bougoffa K, Chibane M, Adach S, Chapeleur Y, Max H, Laurain-Mattar D (2007). Antioxidant activities of alkaloid extracts of two Algerian species of *Fumaria* : *Fumaria capreolata* and *Fumaria bastardii*. Records of Natural Products, 1(2-3): 28-35.
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.
Mizushima Y, Kobayashi M (1968). Interaction of anti -inflammatory drugs with serum proteins, especially with some biologically active proteins, J. Pharm. Pharmacol., 20: 169-173.
Mukherjee PK, Kumar V, Houghton PJ (2007). Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytother. Res., 21: 1142-1145.
Nogala-Kalucka M, Rudzinska M, Zadernowski R, Siger A, Krzyzostaniak I (2010). Phytochemical content and antioxidant properties of seeds of unconventional oil plants, J. Am. Oil Chem. Society, 87(12): 1481-1487.
Neichi T, Koshihara Y, Murota S (1983). Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. Biochim. Biophys. Acta., 753: 130-132.
Neukirch H, D'Ambrosio M, Sosa S, Altinier G, Loggia RD, Guerriero A (2005). Improved Anti-Inflammatory Activity of Three New Terpenoids Derived, by Systematic Chemical Modifications, from the Abundant Triterpenes of the Flowery Plant *Calendula officinalis.* Chem. Biodivers., 2(5): 657-671.
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.
Okoli CO, Akah PA (2004). Mechanism of the anti-inflammatory activity of the leaf extracts of *Culcasia scandens* P. beauv (Araceae). Pharmacol. Biochem. Behav., 79: 473-481.
Oyedepo OO, Femurewa AJ (1995). Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides, Olax subscorpioides* and *Tetrapleura tetraptera*. Int. J. Pharmacog., 33: 65-69.
Parekh J, Chanda SV (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants, Turk J. Biol., 31: 53-58.
Pelczar MJ, Chan ECS, Krieg NR (1988). Control of microorganisms, the control of microorganisms by physical agents. Microbiology. pp. 469- 509.
Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotech., 1: 1142-1145.
Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V (2008). Antimicrobial and antioxidant activity of bioreactive constituents from *Hydnophytum formicarum* Jack. Molecules, 13:904-921.
Prakash A (2001). Anti oxidant activity. Analytical progress, 19(2): 1-6.
Rotimi VO, Laughon BE, Barlett JG, Mosadomi HA (1988). Activities of Nigerian chewing stick extracts against *Bacteroides gingivalis* and *Bacteroides melaninogenicus*. Antimicrob. Agents Chemother,, 32: 598-600.
Roy SP, CM Niranjan TM Jyothi, MM Shankrayya, KM Vishawanath, K Prabhu, VA Gouda, Setty RS(2010). Antiulcer and anti-inflammatory activity of aerial parts *Enicostemma littorale* Blume. Pharmacology, 2(4): 369-373.
Tincusi BM, Jimenez IA, Bazzocchi IL, Moujir LM, Mamani ZA, Barroso JP, Ravelo AG, Hernandez BV (2002). Antimicrobial terpenoids from the oleoresin of the Peruvian medicinal plant *Copaifera pauper*. Planta Med., 68(9): 808-812.
Tomić A, Petrović S, Pavlović M, Trajkovski B, Milenković M, Vučićević D, Niketić D (2009). Antimicrobial and antioxidant properties of methanol extracts of two *Athamanta turbith* subspecies. Pharm. Biol., 47(4): 314–319.
Sadique J, Al-Rqobahs WA, Bughaith, Ar. El-Gindi (1989). The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. Fitoterapia, 60: 525-532.
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. Phytochemistry, 30: 3875-3883.
Sheeja K, Kuttan G (2007). Activation of cytotoxic T lymphocyte responses and attenuation of tumour growth *in vivo* by *Andrographispaniculata* extract and andrographolide. Immunopharm. Immunotoxicol., 29: 81-93.

Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN (1999). Membrane stabilization activity- a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia, 70: 251-257.

Singh B, Singh S (2003). Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. Phytother. 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.

Souza SM, Aquino LC, Milach AC, Jr Bandeira MA, Nobre ME, Viana GS (2007). Anti-inflammatory and antiulcer properties of tannins from *Myracrodruon urundeuva* Allemão (Anacardiaceae) in rodents. Phytother. Res., 21(3): 220-225.

Szollosi R, Szollosi Varga I (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). Acta Biologica Szegediensis, 46: 125-127.

Vane JR, Botting RM (1995). Ne insight into the mode of action of anti-inflammatory drugs. Inflammation Res., 44: 1-10.

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.

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.

Yamamoto Y, Miura Y, Higuchi M, Kinoshita Y, Yoshimura I (1993). Using lichen tissue cultures in modern biology. Bryologist, 96(3): 384-393.

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.