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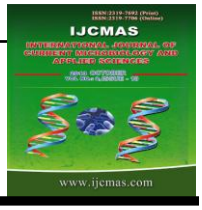

Research Article

Assay of Antibacterial and Antioxidant Activities of Aqueous Leaf Extract of White Ekka (*Calotropis gigantea*)

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A B S T R A C T

The present study was conducted with the main purpose of assay of the antibacterial and antioxidant activities of aqueous leaf extract of White ekka (*Calotropis gigantea*). The leaves of White ekka were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with double distilled water. Antioxidant assay of aqueous (aq.) leaf extract of White ekka was carried out by *in-vitro* model using DPPH free radical scavenging activity. *In-vitro* antibacterial activity of aq. leaf extract of White ekka was assessed using agar well diffusion method with Chloramphenicol as positive control and Dimethyl sulfoxide (DMSO) as negative control, and the zones of inhibition after 48 hours of was measured in millimeters (mm). The antimicrobial activity of aq. leaf extract of White ekka was also evaluated against some pathogenic microorganisms viz. *P. aeruginosa*, *S. aureus*, *K. pneumonia*, and methicillin resistant *Staphylococcus aureus* (MRSA). Results revealed that aq. leaf extracts of White ekka showed the highest scavenging activity (57.14%) at 10 mg/mL and lowest (30.70%) at 2 mg/mL indicating the dose dependent antioxidant activity of aq. leaf extracts of White ekka. Aq. leaf extracts of White ekka possess potential antibacterial activity against *P. aeruginosa*, *S. aureus*, *K. pneumonia*, and MRSA. In conclusion, this preliminary study supplies as evidence-based study for aq. leaf extracts of White ekka could be used alternative to synthetic antioxidants and antimicrobials. However, it needs to be confirmed further with *in vivo* studies.

K e y w o r d s

White Ekka, *C. gigantea*, *Antimicrobial*, *Antioxidant*, *DPPH assay*

Introduction

From prehistorical times to the modern era in numerous parts of the world and India, plants, animals and the natural objects have profound regulate on culture and civilization of man. Plants are one of the most important sources of medicines. They can synthesize different bioactive molecules such as phenols, flavonoids, vitamins, alkaloids, terpenoids, tannins, glycosides, quinones and many others. Most of the plants used for medicinal purposes have been identified and their uses are well documented and described by various research investigators

(Nadkarni, 1976; Dastur, 1985; Saradamma, 1990).

Subsequently the beginning of civilization, human beings have worshiped plants. White ekka (*Calotropis gigantea*) is one such plant. White ekka belongs to *Apocynaceae* family is an improbable shrub reaching 2.4-3m height. White ekka has been cultivated across India in warm dry places from Punjab to western, central and southern India. White ekka is considered as a medicinal plant of India. (Joshi *et al.,* 2011) Plants possess stems, branches, and relatively leaves, mostly condensed near the growing tip (Figure 1). (Sharma *et al.,* 2000)

Free radicals form in our body as a result of biological oxidation. Oxidation is a natural process in organisms to produce energy to fuel biological cycles. Oxidation by-products of normal metabolism cause extensive damage to DNA, protein, and lipids, constituting a major contribution to ageing and to degenerative disease.

Oxidative damage is associated with chronic degenerative diseases, including cancer, coronary artery disease, hypertension, and diabetes (Thomson, 1995). An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism. (Miller *et al.,* 2000)

White ekka is scientifically reported for its anti-candida activity (Gaurav Kumar and Bhaskara Rao, 2010), cytotoxic activity (Wang *et al.,* 2008), antipyretic activity, (Chitme *et al.,* 2005) and wound healing activity Saratha *et al.,* (2009), anti-inflammatory, antiulcer, cytotoxic (Yim *et al.,* 2006), antiproliferative, larvicidal activity, and antidiabetic effect (Kazmi *et al.,* 2012). In the current study we aimed to evaluate the antibacterial and antioxidant activities of aqueous (aq.) leaf extract of White ekka (*C. gigantea*).

Materials and Methods

Collection of plant material

The leaves of White ekka were collected in and around Chikkaballapura district, Karnataka, India. The leaves were sprayed with ethanol, and then shade dried at room temperature for 10 days. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of White ekka were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay (Blois , 1958; Uddin *et al.,* 2012). Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in methanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol

solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation.

Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.,* Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trend line.

Evaluation of Antibacterial Activity

Pathogenic microorganisms

The multiple antibiotic-resistant isolates *viz. Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia,* and methicillin resistant *Staphylococcus aureus* (MRSA) were isolated from clinical samples of local hospital in and around Chikkaballapura and confirmed by various microscopic evaluation like Gram's staining (Gram, 1884). Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne (1970). All the bacterial pathogens were further confirmed by suitable biochemical tests (Barrow and Feltham, 1993), and used for antimicrobial activity studies.

The direct colony suspension method is the most convenient method for inoculum preparation. The inoculum was prepared by making a direct broth or saline suspension of isolated colonies selected from an agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 × 10^8 colony-forming units (CFU)/mL. To perform this step accurately, used adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, the pathogenic bacteria culture was inoculated into culture plates to screen for antibacterial properties.

Determination of Antibacterial Activities

Antibacterial activity of aq. leaf extract of White ekka was tested by agar well diffusion method (Khyade and Vaikos, 2009). The culture plates were prepared by pouring 20 ml of sterile Muller Hintonagar (MHA). 1 ml inoculums suspension was spread uniformly over the agar medium using a sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (6 mm) was used to make wells in each plate for extracts.

These plates were labeled and aq. leaf extract of *C. gigantea* plant (at a concentration of 100, 50, 25, 12.5mg/ml) was added aseptically into the well. Also, 5% DMSO and chloramphenicol (10µg) were used as negative and positive control respectively. Plates containing drug were left for one hour in order to diffuse properly in media and to get dry. Then the plates were incubated for 24 h at 37°C during which the activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of the diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

Results and Discussion

Antioxidant activity

The aq. leaf extracts of White ekka showed scavenging activity against the free radicals. The aq. leaf extracts of White ekka showed the highest scavenging activity (57.14%) at 10mg/mL and lowest (30.70%) at 2mg/mL (Table 1). It was found that when the concentration of the extract increased, the absorbance value gets decreased regularly. The presence of unpaired electron imparts a strong absorbance at 517 nm, giving the radical a purple color. With the exposure to antioxidants, it undergoes reduction, decreasing absorbance due to the formation of yellow colored anti-radical diphenyl picryl hydrazine (DPPH). The degree of colour change from purple to yellow is a measure of scavenging potential of the antioxidants in the extracts in terms of hydrogen donating ability (Sre *et al.,* 2012).

Antibacterial activity

The antibacterial activity of aq. leaf extracts of White ekka is tested against different bacterial strain. All the bacterial strain were treated with different concentration of aq. leaf extracts of White ekka (12.5 mg/mL, 25 mg/ mL, 50 mg/ mL, and 100 mg/ mL) and 5% DMSO as negative control and 10 microgram chloramphenicol as positive control. Antibacterial activities of all the four different concentrations against selected bacterial strains were recorded in the form of zone of inhibition and measured in millimeter (mm). The highest zone of inhibition (16.2 mm) was observed against MRSA and lowest zone of inhibition (12.6 mm) was observed against *P. aeruginosa* at 100 mg/mL. At 50 mg/mL the highest zone of inhibition (14.2mm) was observed against *S. aureus* and lowest zone of inhibition (11.2 mm) was observed against *P. aeruginosa*. Whereas the highest zone of inhibition (10.9 mm) was seen against *S. aureus* and lowest zone of inhibition (6.9 mm) against *K. pneumonia* at 25 mg/mL. At 12.5 mg/mL the highest zone of inhibition (9.2 mm) was observed against *S. aureus* and lowest zone of inhibition (1.5 mm) was observed against *K. pneumoniae.* The reference standard Chloramphenicol showed highest zone of inhibition (22.5 mm) against MRSA and the lowest zone of inhibition (15.6mm) against at 10 µ/mg *K. pneumoniae.* These findings revealed that the tested aq. leaf extracts of White ekka possess potential antibacterial activity against *P. aeruginosa, S. aureus, K. pneumoniae*, and MRSA as shown in Table 2.

Earlier studies on the antimicrobial activity of White ekka extracts revealed its antibacterial potential against *Sarcina lutea, Bacillus. megaterium, Bacillus. subtilis, Shigella sonnei, E. coli* and *P. aeruginosa* (Alam *et al.,* 2008). Literature reports evidenced that the presence of phytochemicals like cardenolides, flavonoids, terpenes, nonprotein amino acid and cardiac glycoside as major constituents in White ekka may be responsible for the antibacterial activity of this plant. (Ali and Gupta, 1999; Wang *et al.,* 2008)

The results of present study delineated that aq. leaf extracts of White ekka exhibited effective antioxidant and antibacterial properties. These biological activities of aq. leaf extracts of White ekka could attributable to phytoactives like cardenolides, flavonoids, terpenes, nonprotein amino acid and cardiac glycoside present in it.

Table.1 Antioxidant activity of aq.leaf extract of White ekka

Concentration of Aq. Extract White ekka (mg/mL)	DPPH Scavenging Activity (%)
2	30.70
4	36.37
6	45.03
8	51.41
10	57.14

Table.2 Antibacterial activities of aq. leaf extracts of White ekka

Bacterial strains	Zone of inhibition(mm)					
			Aq. leaf extracts of White ekka			
	Negative Control	Positive Control	12.5 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL
P. aeruginosa	-	17.2	7.2	8.9	11.4	12.6
S. aureus	-	18.9	9.2	10.9	14.2	15.9
K. pneumonia	-	15.6	1.5	6.9	11.2	12.9
MRSA	-	22.2	7.2	9.6	13.2	16.2

Figure.1 Showing White ekka (*C. gigantea*) plant

Therefore, this preliminary study supplies as evidence-based study for aq. leaf extracts of White ekka could be used alternative to synthetic antioxidants and antimicrobials since aq. leaf extracts of White ekka mimic the biological activities of synthetic antioxidant and antimicrobials. However, dosage and safety & toxicity studies are recommended to carry out *in-vivo* for successful therapeutic modality.

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