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ANTIBACTERIAL AND RADICAL SCAVENGING ACTIVITY OF SELECTED PLANTS OF WESTERN GHATS OF KARNATAKA, INDIA

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ABSTRACT

In the present study, we determined the antibacterial and radical scavenging efficacy of extracts of five plants *viz*., *Psychotria nigra* (Gaert.) Alston, *Flacourtia montana* Graham, *Aglaia roxburghiana* (W.&.A) Miq. Var. *beddomei*, *Canthium dicoccum* (Gaertn.) Teys. & Binn. and *Ligustrum roxburghii* C.B. Clarke collected at Haniya, Western Ghat region of Hosanagar Taluk, Shivamogga district, Karnataka. The leaves were shade dried, powdered and extracted using methanol. Antibacterial activity was determined by agar well diffusion assay against two Gram positive and two Gram negative bacteria. Radical scavenging potential of extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Total phenolic content of extracts was estimated by Folin-Ciocalteau Reagent (FCR) method. The extracts were able to inhibit at least one of the test bacteria. Marked inhibitory activity was observed in case of *L. roxburghii* whereas *C. dicoccum* displayed least inhibitory effect. Extracts were shown to exhibit dose dependent scavenging of DPPH radicals. Marked radical scavenging potential was observed in case of *A. roxburghiana*. A positive correlation was observed between total phenolic content and radical scavenging efficacy of extracts. In conclusion, the plants have been shown promising for the development of pharmacologically active drugs. Further *in vivo* studies are to be conducted.

KEY WORDS: Western Ghats, Antibacterial, Agar well diffusion, Antioxidant, DPPH, FCR

1. INTRODUCTION

Infectious diseases have devastated mankind throughout history. Pathogens such as bacteria, fungi, viruses and parasites are responsible for causing morbidity as well as mortality. These pathogens cause millions of deaths worldwide especially in developing and under-developing countries. Therapy of diseases using antibiotics reduces the morbidity and mortality caused by infectious agents. The antibiotics face a major challenge by the emergence of pathogens which have developed resistance against them. Bacteria such as *S.aureus*, *Streptococcus pneumoniae*, *Enterococcus* spp., *Acinetobacter baumannii*, *K. pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* are among the important drug-resistant bacteria. Moreover, the tendency of pathogens to disseminate the resistance gene is another great threat for disease control. The antibiotic resistance is considered as a global problem and is growing day by day (Yoshikawa, 2002; Lister, 2009; Davies and Davies, 2010; Kekuda, 2013).

Oxidative stress results when the balance between free radical generation and antioxidant defense is disturbed due to excessive production of free radicals or a deficit in antioxidant defense. During oxidative stress, macromolecules such as proteins, lipids and nucleic acids suffer from potential damage and hence oxidative stress is implicated in several diseases or disorders. In the body, the potential harmful effects of these free radicals are nullified by a team of substances termed antioxidants. These antioxidants may be endogenous or obtained from external sources such as diet or medicinal plants. Antioxidants act at different levels *viz*., prevention, interception and repair. Synthetic antioxidants *viz*., Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and Propyl gallate (PG) are widely used as antioxidants but are suspected to be carcinogenic and mutagenic on chronic consumption. Therefore, discovery of safe and effective antioxidants from natural sources is of immense interest. Studies have indicated that consumption of fruits, vegetables, nuts, seeds, whole grains can result in reduced risk of chronic diseases associated with oxidative stress. The protective effect of plants is attributed to the presence of phytochemicals with antioxidant efficacy (Diplock, 1998; Devasagayam, 2003; Dixit, 2005; Bektasoglu, 2006; Choi, 2007; Junaid, 2013).

The rich floristic diversity of India represents about 11% of total world flora. Western Ghats of India is considered as one among the 34 global biodiversity hotspots. The mountain ranges of Western Ghats run through

various states *viz*., Gujarat, Karnataka, Tamil Nadu, Maharashtra, Goa and Kerala and harbors a large number of plant species with high degree of endemism. Various vegetation types such as wet evergreen forests, moist and dry deciduous forests, montane forests, sholas, scrubs and savannas are found in Western Ghats (Aravind, 2007; Richard and Muthukumar, 2012). The central Western Ghats of Karnataka, known as 'Sahyadri', represents a long mountain chain along the west coast of India and encompass districts namely Chikmagalur, Shivamogga, Udupi, Dakshina Kannada, Uttara Kannada, Hassan and Coorg. The present study was conducted with an aim of determining antibacterial and radical scavenging efficacy of extracts of five plants *viz*., *Psychotria nigra*, *Flacourtia montana*, *Aglaia roxburghiana*, *Canthium dicoccum* and *Ligustrum roxburghii* collected at a place called Haniya, Western Ghat region of Hosanagara Taluk of Shivamogga district, Karnataka.

2. MATERIALS AND METHODS

2.1. Collection and identification of plants: The plants (Table.1) were collected at Haniya, Western Ghat region of Hosanagara Taluk of Shivamogga district, Karnataka during January 2014. The plants were identified by Dr. Vinayaka K.S, Department of Botany, KFGC, Shikaripura, Karnataka.

Table.1.Plants used in this study

Plant name	Family	Habit	Reported bioactivities
P.nigra	Rubiaceae	Shrub	Insecticidal (Hewage, 1997), antimicrobial (Jayasinghe, 2002; Kambar, 2014)
F.montana	Flacourtiaceae	Tree	Antifungal (Kambar, 2014)
A.roxburghiana	Meliaceae	Tree	Cytotoxic (Islam, 2009), anti-inflammatory (Janaki, 1999), antifungal (Janaki and Vijayasekaran, 1998; Kambar, 2014)
C.dicoccum	Rubiaceae	Tree	Antidiabetic (Santhan, 2013), nephroprotective (Santhan, 2013), anti-inflammatory (Vuyyuri, 2013), antimicrobial (Jayasinghe, 2002; Kambar, 2014), anti-arthritic (Reddy, 2013)
L.roxburghii	Oleaceae	Tree	Antifungal (Kambar, 2014)

2.2. Extraction: The leaves were separated from plants, washed, dried under shade and powdered mechanically. 25g of each of the leaf material was transferred into separate conical flasks containing 100ml of methanol (HiMedia, Mumbai). The flasks were left for 2 days with occasional stirring. The contents were filtered through Whatman No. 1 filter paper and evaporated to dryness (Manasa, 2013).

2.3. Antibacterial activity of leaf and bark extract: Agar well diffusion assay was performed to determine the efficacy of extracts of selected plants to inhibit bacteria *viz*., *Staphylococcus aureus* NCIM-2079, *Bacillus cereus* NCIM-2016, *Salmonella typhi* MTCC-734 and *Klebsiella pneumoniae* NCIM-2957. 24 hours old Nutrient broth (HiMedia, Mumbai) cultures of test bacteria were swabbed on sterile Nutrient agar (HiMedia, Mumbai) plates followed by punching wells of 6mm diameter using a sterile cork borer. Then, 100µl of extracts (20mg/ml of 25% DMSO [Dimethyl sulfoxide; HiMedia, Mumbai]) of selected plants, reference standard (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were added to labeled wells. The plates were incubated at 37^{o}C for 24 hours in upright position and the zones of inhibition formed were measured using a ruler (Kekuda, 2013).

2.4. Radical scavenging efficacy of leaf and bark extract: The radical scavenging nature of extracts was evaluated by DPPH free radical scavenging assay. Here, 2ml of different concentrations of extracts and reference standard ascorbic acid (6.25-100µg/ml of methanol) was mixed with 2ml of DPPH solution (0.002% in methanol) in clean and labeled tubes. The tubes were incubated for 30 minutes at room temperature in dark. The absorbance was measured at 517 nm using UV-Visible spectrophotometer (ELICO, SL159). The absorbance of the DPPH control (2ml of DPPH+2ml of methanol) was noted. The radical scavenging activity of each concentration of extracts was calculated using the formula:

Scavenging activity (%) = [(A–B) /A] x100, where A is absorbance of DPPH and B is absorbance of DPPH and extract/standard combination. The IC_{50} value for the extract was calculated. IC_{50} represents the concentration of extract required to scavenge 50% of DPPH radicals (Kekuda, 2013).

2.5. Total phenolic content of leaf and bark extract: Folin-Ciocalteu reagent (FCR) method was carried out to estimate the content of total phenolics in the extracts of selected plants. A dilute concentration of extract (0.5ml) was mixed with 0.5ml F-C reagent (1:1) and 2 ml of sodium carbonate (7%). The tubes were left at room

temperature for 30 minutes. The absorbance was measured at 765nm in UV-Visible spectrophotometer (ELICO, SL159).A standard curve was plotted using Gallic acid (standard, 0-1000µg/ml). The content of total phenolics in extracts was expressed as µg Gallic acid equivalents (GAE) from the graph (Kekuda, 2013).

3. RESULTS

3.1. Inhibitory activity of extracts of selected plants: Table 2 shows the result of inhibitory potential of extracts of selected plants against Gram positive and Gram negative bacteria. All bacteria were found to be susceptible to at least one of the extracts. High and least inhibitory efficacy was shown by extract of *L. roxburghii* and *C. dicoccum* respectively. *K. pneumoniae* was least inhibited by extract of selected plants. Chloramphenicol inhibited test bacteria to higher extent when compared to extracts. No inhibitory activity was observed in case of DMSO.

Table.2.Inhibitory effect of extracts of selected plants against bacteria

Treatment	Zone of inhibition in cm			
	S. aureus	***B. cereus***	***S. typhi***	***K. pneumoniae***
P. nigra	1.3±0.0	1.0±0.0	1.5±0.1	1.0±0.0
F. montana	1.3±0.1	1.6±0.1	1.3±0.0	0.0±0.0
A. roxburghiana	1.5±0.1	0.8±0.0	1.2±0.0	0.8±0.0
C. dicoccum	1.1±0.0	0.8±0.0	1.0±0.0	0.0±0.0
L. roxburghii	2.1±0.1	2.9±0.1	1.9±0.1	1.7±0.1
Chloramphenicol	3.1±0.1	3.0±0.2	2.6±0.2	2.7±0.1
DMSO	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

3.2. Radical scavenging efficacy of extracts of selected plants: The result of radical scavenging potential of extracts is shown in Figure 1. All extracts scavenged DPPH radicals in a dose dependent manner. Highest and least radical scavenging activity was observed in case of *A. roxburghiana* and *F. montana* respectively. The IC_{50} for *P. nigra*, *F. montana*, *A. roxburghiana*, *C. dicoccum* and *L. roxburghii* was found to be 52.12, 59.01, 19.94, 49.32 and 53.59µg/ml respectively. Ascorbic acid scavenged free radicals to higher extent (IC_{50} 2.49µg/ml) when compared to extracts of selected plants.

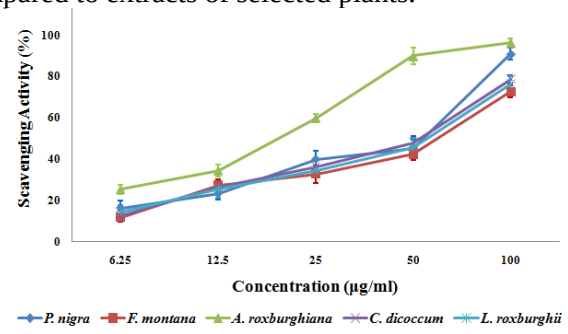

Figure 1: Scavenging of DPPH radicals by extracts of selected plants

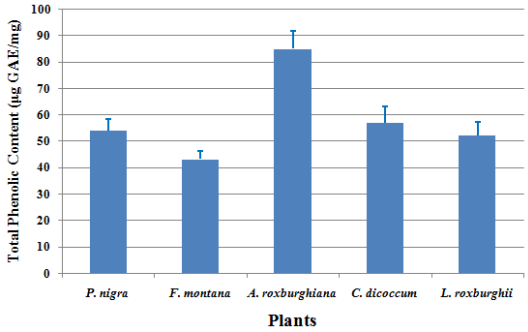

Figure 2: Content of total phenolics in extracts of selected plants

3.3. Total phenolic content of extracts of selected plants: The content of total phenolics of extracts of selected plants ranged between 43.28 and 85.10µg GAE/mg. Phenolic content was found to be higher in *A. roxburghiana* (85.10µg GAE/mg) while *F. montana* (43.28µg GAE/mg) was shown to contain lesser phenolic content among extracts of selected plants (Figure.2).

4. DISCUSSION

Plants have been used for the treatment of diseases since time immemorial. Plants constitute a key component of traditional medicine as they are important source of valuable medicines. Several countries of Africa, Asia and Latin America depend on traditional medicine. It is estimated that >80% of world's population utilize traditional medicine in order to meet primary health care needs. All over the world, folk healing commonly utilize herbs as part of tradition. The practice of plant based traditional medicine is widespread in several countries *viz*., China, India, Japan, Pakistan, Sri Lanka, Thailand etc. Plants have been extensively used in various systems of traditional medicine such as Ayurveda, Unani, Homeopathy and Sidda. Often, these plants provide

lead compounds for developing pharmacologically active drugs. Drugs such as aspirin, digoxin, quinine and morphine are derived from plants (Pal and Shukla, 2003; Kassaye, 2006; Mazid, 2012; Kekuda, 2013).

The search for alternative disease therapy has been intensified due to high cost of antibiotics, side effects associated with their use and the emergence of antibiotic resistant pathogens. Plants are considered as promising alternatives for treatment of infections caused by pathogens including drug resistant strains (Dahiya and Purkayastha, 2012; Manasa, 2013; Kekuda, 2014). In the present study, we screened antibacterial effect of five plants from Western Ghats of Karnataka, India. Among the selected plants, marked inhibitory potential was shown by *L. roxburghii*. Least antibacterial effect was shown by *C. dicoccum*. All extracts were effective against *S. aureus*, *B. cereus* and *S. typhi*. However, extract of *F. montana* and *C. dicoccum* were not inhibitory to *K. pneumoniae*. In a study, Jayasinghe et al. (2002) found higher inhibitory activity of bark extracts than leaf extracts of *C. dicoccum* and *P. nigra*. Recently, Kambar et al. (2014) showed potential of the plants selected in the present study to inhibit the mycelial growth of *C. capsici* isolated from chilli anthracnose.

Several *in vitro* assays are used to evaluate radical scavenging potential of samples. Among them, DPPH free radical scavenging assay is one of the most widely used assays. DPPH is a stable, nitrogen centred, organic free radical having absorption maximum at 517nm in alcoholic solution. The radical becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom from antioxidant. In the presence of a donor capable of donating hydrogen atom, the radical nature of DPPH is lost and its color (purple) changes to yellow (diphenylpicrylhydrazine). The solution loses color stoichometrically depending on the number of electrons taken up. This assay has been used to study radical scavenging activity of various kinds of samples including plant extracts (Devasagayam, 2003; Chung, 2006; Kekuda, 2011; Seruga, 2011; Kekuda, 2013). In the present study, the decrease in absorption of DPPH in the presence of varying concentrations of extracts of selected plants was monitored at 517nm. Marked scavenging efficacy was observed in case of extract of *A. roxburghiana* while extract of *F. montana* displayed least scavenging activity. The scavenging effect of extracts was lesser than that of ascorbic acid. Although the scavenging potential of extracts were lesser than that of ascorbic acid, it is clear from the study that the extracts possess hydrogen donating ability and hence, these extracts can act as free radical scavengers, acting possibly as primary antioxidants (Chung, 2006).

Plants produce a variety of secondary metabolites. Among these, polyphenols (including flavonoids) are a large and diverse group of plant metabolites and are known to possess a wide array of biological activities including antioxidant activity. Hence, it is important to estimate total phenolic contents of plant extracts so as to justify their contribution to antioxidant activity. In the present study, the total phenolic content of extracts of selected plants was estimated by FCR method. FCR method is one of the commonly used and oldest colorimetric methods for estimating phenolic constituents of different kinds of substances including plant extracts. The phenolic compounds react with FCR under basic conditions and form blue complex with absorption maxima at around 750nm. The assay is convenient, simple, and reproducible and hence, it has become a routine assay for studying the phenolic antioxidants (Chung, 2006; Choi, 2007; Coruh, 2007; Kekuda, 2011; Rekha, 2012; Junaid, 2013). The phenolic content was found to be maximum and minimum in extract of *A. roxburghiana* and *F. montana* respectively. Earlier studies have shown a direct correlation between phenolic contents and radical scavenging activity (Tilak, 2004; Coruh, 2007; Rekha, 2012; Poornima, 2012; Kekuda, 2013). In the present study also, extracts with high phenolic contents displayed stronger radical scavenging activity.

5. CONCLUSION

In conclusion, the plants selected in this study exhibited antibacterial and radical scavenging activity. The plants can be used against infectious agents and oxidative stress. The plants can be exploited for the development of drugs with pharmacological activities. Further studies concerning isolation of active constituents from extracts and determination of their bio-efficacies are to be conducted.

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