

Phytochemical Screening, Antimicrobial and Antioxidant Activities of Aerial Parts of *Quercus robur* L.

Ghias Uddin and Abdur Rauf

Institute of Chemical Sciences, Centre for Phytomedicine and Medicinal Organic Chemistry,
University of Peshawar, Peshawar, KPK, 25120, Pakistan

Abstract: Phytochemical screening is an important step for the isolation of bioactive chemical constituents. *Quercus robur* L. aerial parts have been selected to analyse for bioactive natural products. The phytochemical screening showed the presence of different classes of bioactive secondary metabolites. The *in vitro* antimicrobial activity of hexane, chloroform, ethyl acetate and methanolic fraction of *Q. robur* were evaluated against selected pathogens showing moderate activities against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Staphylococcus epidermidis* and *Bacillus subtilis* while antioxidant activity (2,2-diphenyl-1-picrylhydrazyl radical scavenging assay) indicated moderate scavenging activity among the entire fractions.

Key words: *Quercus* Linn • Phytochemical screening • Antibacterial activity • Antioxidant activity

INTRODUCTION

Medicinal plants are a rich source of producing wide number of chemical constituents in most efficient way and with precious selectivity. According to the World Health Organization more than 80% of the world's people depend on traditional medicine for their primary healthcare needs. The medicinal value of the plants is due to their chemical constituents that bring a definite physiological action on the human body to prevent from the diseases. In our continuing studies on medicinal plants; need to screen a number of medicinal plants for promising biological activity [1]. In the recent years, phytochemicals in fruits and vegetables have received a great deal of attention mainly to prevent human diseases caused by oxidative stress which releases reactive oxygen species involved in a number of disorders such as cardiovascular breakdown, tissue injury, DNA damage and develop such tumor. Several studies suggest that antioxidant could prevent accumulation of these reactive oxygen species and be beneficial for the treatment of these pathologies. Human diets which contain vegetables and fruits reduced risk of a variety of tumors, especially epithelial cancers of respiratory and gastrointestinal tract. The systemic screening of antimicrobial plant extracts represents a continuous effort to explore new biologically active

chemical constituents to act against multi-resistant pathogenic bacteria. As a part of our research studies on plants, one of the well know *Quercus* (Fagaceae) species is *Q. Robur* which has been used for the treatment of various diseases such as haemorrhages, chronic diarrhoea, dysentery and asthma. Certain species of *Quercus* are used for treatment of gonorrhoea, diarrhoea, indigestion, asthma, astringent, anti-diabetic, anti-tremor, local anaesthetic, antipyretic, anti-inflammatory, anti-Parkinson and hepatoprotective [2-4]. The present study deals with preliminary phytochemical screening and biological evaluation of aerial parts of *Q. robur* L.

MATERIALS AND METHODS

Collection of Plant Material: *Quercus robur* aerial parts were collected from Razagram District Dir, Khyber Pakhtunkhwa, Pakistan, during the month of February, 2011. The plant was recognized by Prof. Dr. Abdur Rashid and a voucher specimen (No. R.A 763) was deposited in the herbarium, Department of Botany, University of Peshawar, Peshawar, Pakistan.

Extraction and Fractionation of Plant Material: Shade dried aerial parts of *Q. robur* was successively extracted with methanol (x3). The combined methanolic

extract was concentrated under vacuum into thick syrup and fractionated into n-hexane, chloroform and ethyl acetate fractions.

Micro-Organism Assortment and Preservation: Three selected strains of Gram-positive bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*) and two of Gram-negative bacteria (*Klebsiella pneumonia* and *Escherichia coli*) were obtained from stock culture PNRL laboratories, Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan and stored in Müller-Hinton agar at low temperature (4°C) prior to subculture.

Antimicrobial Assay against Selected Bacterial Strains: Modified agar well diffusion method was adopted to test the antibacterial activity of the fractions with the use of Müller-Hinton agar (MHA) as medium. The cultures were equipped in triplicates at 37°C incubation temperature for a period of 24 to 72 hours. 0.6 ml of the broth culture of the tested organism was put in a sterile Petri-dish and added 20 ml of the sterile molten MHA. Wells were made into the medium using 0.2 ml of each fraction while Streptomycin (2 mg/ml) was used as a standard of the antimicrobial agent. Inoculation was done for an hour ensure the diffusion of the antimicrobial agent into the medium. The inoculation plates were incubated for 24 hour at 37°C and the diameters of the zone of inhibition of microbial growth were measured in millimetres.

Antioxidant Activity: The hydrogen atom or electron donation abilities of the corresponding extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH.) Assay was carried out according to the standard method of Blois [5, 6] with a slight modification. Briefly, a 1 mM stock solution of DPPH radical solution in methanol was prepared and 1 ml of stock solution was mixed with 3 ml of sample solutions in ethanol (containing 20-100 µg) and control (without sample). The resultant mixture was briefly shaken and maintained at room temperature for 30 min. Absorbance was measured spectrophotometrically at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Radical scavenging activity (RSA) was expressed as the inhibition percentage and calculated by using the following equation,

$$\text{RSA \%} = [(A-B)/A] \times 100$$

where,

A = Absorbance of the control solution and

B = Absorbance of the test solution.

Phytochemical Screening: The phytochemical tests were performed to assess the qualitative chemical composition of different fractions of *Q. robur* followed by the standard procedures illustrated by [7-10] for detection of the phytochemicals.

Alkaloids: 0.2 g of each fraction was warmed with 2% H₂SO₄ for two minutes. The reaction mixtures were filtered and added few drops of Dragendorff's reagent to each filtrate. Orange red precipitate indicates the presence of alkaloids moiety.

Tannins: A small quantity of each extract was mixed with water and heated on water bath then filtered. A few drops of ferric chloride were added to each filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones: 0.5 g of each extract was boiled with 10 % HCl for few minutes on water bath. The reaction mixtures were filtered and allowed to cool. Equal volume of CHCl₃ was added to each filtrate and few drops of 10% ammonia was also added to each reaction mixture and heated for few minutes. Rose-pink colour formation indicates the presence of anthraquinones.

Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. Few drops of Fehling's solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Reducing Sugars: Each extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Fehling, s solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Saponins: 0.2 g of each extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

Flavonoids: 0.2 g of each extract was dissolved in diluted NaOH and few drops of conc. HCl were added. A yellow solution that turns colourless indicates the presence of flavonoids.

Table 3: Antibacterial activity of *Q. robur* fractions

| Microorganism | Gram | Hexane | CHCl ₃ | EtOAc | MeOH | Streptomycin |
|-----------------------------------|------|--------|-------------------|-------|------|--------------|
| <i>Escherichia coli</i> | - | 0 | 14 | 14 | 12 | 28 |
| <i>Staphylococcus aureus</i> | + | 0 | 0 | 16 | 18 | 30 |
| <i>Klebsiella pneumonia</i> | - | 0 | 12 | 12 | 0 | 30 |
| <i>Staphylococcus epidermidis</i> | + | 18 | 20 | 20 | 0 | 28 |
| <i>Bacillus subtilis</i> | + | 0 | 14 | 12 | 0 | 0 |

Key word: Well size: 6mm

CONCLUSIONS

It has been concluded that *Q. robur* contained interesting biological active phytoconstituents which exhibited moderate antimicrobial and antioxidant activity. After toxicological studies on some eventual harmful chemical constituents present in the extracts or their fractions and suggest using plant extract as natural antioxidant and antimicrobial for nutraceutical.

REFERENCES

- Asmita, D. and S.S. Mukesh, 2011. Comparative analysis of aqueous extracts of amaranth and coriander in scavenging free radical activity and protection of DNA against oxidative damage, *Chiang Mai Journal of Science*, 38: 560-571.
- Haq F., H. Ahmad and M. Alam, 2011. Traditional uses of medicinal plants of Nandiar Khuwarr catchment (District Battagram), Pakistan, *Journal of Medicinal Plants Research*, 5: 39-48.
- Khadem, S. and R.J. Marles, 2010. Monocyclic phenolic acids; hydroxy- and polyhydroxybenzoic acids: occurrence and recent bioactivity studies, *Molecules*, 15: 7985-8005.
- Aroonrerk, N. and N. Kamkaen, 2009. Anti-inflammatory activity of *Quercus infectoria*, *glycyrrhiza uralensis*, *kaempferia galangal* and *coptis chinensis*, the main components of Thai herbal remedies for aphthous ulcer, *Journal. Health Research*, 23: 17-22.
- Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical, *Nature*, 26: 1199-1200.
- Yıldırım A., A. Mavi and A.A. Kara, 2003. Antioxidant and antimicrobial activities of *Polygonum cognatum* Meissn extracts, *Journal of Science Food Agriculture*, 83: 64-69.
- Uddin, G., A. Rauf, M. Arfan, M. Ali, M. Qaisar, M. Saadiq and M. Atif, 2012. Preliminary phytochemical Screening and antioxidant activity of *Bergenia Caliata*. *Middle-East Journal of Scientific Research*, 11: 1140-1142.
- Uddin, G., W. Ullah, A. Rauf, B.S. Siddiqui, T.U. Rehman, S. Azam and M. Qaisar, 2011. Phytochemical screening and antimicrobial activity of *Cornus microphylla* Wall, ex Roxb, 2011, *Middle-East Journal of Scientific Research*, pp: 516-519.
- Uddin, G., A. Rauf, M. Qaisar, A. Latif and M. Ali, 2011. Preliminary Phytochemical Screening and Antimicrobial Activity of *Hedera helix* L, *Middle-East Journal of Scientific Research*, 8(1): 198-202.
- Uddin, G., A. Rauf, T.U. Rehman and M. Qaisar M, 2011. Phytochemical screening of *Pistacia chinensis* var. *integerrima*, *Middle-East Journal of Scientific Research*, 7: 707-711.
- Chung, K.T., Y.W. Huang and Y. Lin, 1998. Tannins and human health; a review, *Critical Reviews in Food Science and Nutrition*, 38: 421-464.
- Price, K.R., T.I. Johnson and G.R. Fenwick, 1987. The chemistry and biological significance of saponins in food and feeding stuffs, *Critical Reviews in Food Science and Nutrition*, 26: 22-48.
- Sayyah, M., N. Hadidi and M. Kamalinejad, 2004. Analgesic and anti-inflammatory activity of *lactuca sativa* seed extract in rat, *Journal of Ethnopharmacology*, 92: 325-329.
- Malairajan, P., G. Gopalakrishnan, S. Narasimhan and K.V.K. Jessi, 2006. Analgesic activity of some Indian medicinal plants, *Journal Ethnopharmacology*, 106: 425-428.
- Rupasinghe, H.P. C.J. Jackson, V. Poysa, C. DiBierado, J.D. Bewley and J. Jenkinson, 2003. Soyasapogenol A and B distribution in glycin max in relation to seed physiology, genetic variability and growing location, *Journal Agriculture Food Chemistry*, 51: 5888-5894.
- Polterait, O., 1997. Antioxidant and free-radical scavengers of natural origin, *Current Organic Chemistry*, 1: 415-440.