Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata* (Lour.) Stem Bark

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ABSTRACT

Background: Stem barks of *Ficus auriculata* have been used in Nepal as folk remedy in the form of juice to treat diarrhea, dysentery, cuts and wounds. So this study is designed to explore the antioxidant and antibacterial activity of *F. auriculata* stem bark. **Methods:** Stem barks were subjected to successive extraction using hexane, chloroform and methanol to obtain the respective extracts. DPPH free radical scavenging method was used for antioxidant activity analysis. Agar well diffusion method was used for the antibacterial activity test. **Results:** Qualitative phytochemical analysis of methanol extracts of *F. auriculata* stem bark showed the presence of alkaloids, carbohydrates, saponins, glycosides, phytosterols, resins, phenols, tannins, diterpenes, flavonoids, proteins, and amino acids. Antioxidant activity of methanol extract was found to be 84.088% at 0.1 mg/ml with IC_{50} value of 0.042 mg/ml ($r^2 = 0.9942$) and that of chloroform extract was found to be 83.864% with IC_{50} value of 0.029 mg/ml ($r^2 = 0.955$). However free radical scavenging activity of hexane extract was of 42% ($r^2 = 0.9036$) in comparison of standard ascorbic acid used. Antibacterial activity of methanol extract was higher for *Escherichia coli* with 4.5±0.15 mm zone of inhibition and for *Staphylococcus aureus* hexane extract was highly effective with the zone of inhibition of 7.8±0.36 mm. **Conclusion:** Plant extract showed potential antioxidant activity but antibacterial activity was found to be comparatively lower to that of the standard antibiotics used.

Key words: ***F****icus auriculata*, **p**hytochemical screening, **a**ntibacterial activity, **a**ntioxidant activity, **D**PPH

INTRODUCTION

Ficus, the fig genus, consists of over 800 species in 40 genera of the mulberry family, Moraceae. A number of *Ficus* species are used as food and for medicinal properties in Ayurvedic and Traditional Chinese Medicine (TCM) to treat several common ailments. Thirty-six species of *Ficus* are reported so far from Nepal but the detail investigation of their indigenous uses have never undertaken till now.[1] *Ficus auriculata* Lour. is widely distributed in temperate, tropical and subtropical regions of about 1800 – 2600 m altitude.[2] It is tree of about 4 -10 m tall and dioecious in nature. It contains abundant amount of white latex in every part of the plant.[3] Bark is grayish brown with rough texture. Branchlets are reddish brown. Figs (also called as fruits) are reddish brown, pear-shaped, depressed globose or top-shaped in nature and generally occur on leafless branchlets at base of trunk and main branches.[4-5]

The ethnomedicinal and traditional uses of *F. auriculata* in the treatment of diarrhea, dysentery, cuts, wounds, mumps, cholera, jaundice[6] etc. suggest that the plant must have antimicrobial as well as antioxidant efficacy. Several studies on other species of *Ficus* have shown the potential antioxidant and antimicrobial activity, but clinical researches regarding these properties of *F. auriculata* have not been carried out. So our main aim in this research is to explore the antibacterial and antioxidant efficacy of different extracts of bark of *F. auriculata*. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[7,8] That's why before investigating pharmacological activity of plant extracts preliminary phytochemical screening plays the pivotal role.

Antioxidant defense system combats against wide range of degenerative diseases including inflammation, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson,

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and coronary heart pathologies resulted due to the free radicals and oxidative stress.[9-11] The main cause of oxidative stress in the body includes free radicals namely, superoxide ($O_2^{-\cdot}$), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$), peroxynitrite ($^{\cdot}ONOO^{\cdot}$) and nitric oxide ($NO^{\cdot}$) radicals as well as non free radical species as hydrogen peroxide (H_2O_2), nitrous acid (HNO_2) and hydrochlorous acid (HOCl).[12-13] Reactive oxygen species (ROS) can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids and lipoprotein by propagating a chain reaction cycle. Thus, antioxidants are coevolved with aerobic metabolism to counteract oxidative damage from ROS.[14-16]

Nowadays lots of synthetic antioxidants are subjected in Pharma markets by several manufacturers but these synthetic antioxidants possess greater risks of side effects; therefore, investigations on identifying the natural antioxidants have become very important issue.[17] In the past few years, natural antioxidants have generated considerable interest in preventive medicine. Plants produce a huge amount of antioxidants and they can represent a potential source of new compounds having antioxidant properties with fewer side effects.[18-19]

The screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes. Contrary to the synthetic drugs, antibacterial activities of phytochemicals are associated with lesser side effects and have an enormous therapeutic potential to heal many infectious diseases.[20] The potential for developing antibacterial from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Nowadays a number of clinically efficacious antibiotics are becoming less effective due to development of resistance and this has caused serious clinical problems in the treatment of infectious diseases.[21-24] So, biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens.[25]

MATERIALS AND METHODS

Plant collection and authentication

The plant barks were collected from the forest of Lekhnath municipality of Kaski district of Nepal and properly identified and confirmed in the department of Pharmacognosy of Pokhara University, where voucher specimen were deposited with the number HN 1294. The crude stem bark sample was deposited into the crude drug preservation laboratory with SN 387. Bark were thoroughly cleaned with tap water, lichens on the outer surfaces were removed by scrapping with stainless still knife, cut into pieces of dimension about 1.5 – 2 × 1 × 0.5 cm^3 and shed dried till complete dryness [Figure 1].

Other materials in experiment

Gram positive, *Staphylococcus aureus*, and gram negative *Escherichia coli*, used in the study, were obtained from the microbiology department of WHO – GMP certified Nepal Pharmaceuticals Laboratory Pvt. Ltd, Birgunj, Nepal. All chemicals used in the research purposes were obtained from the Merck and Qualigens fine chemicals. They were of analytical grade.

Preparation of extract

4 kg of dried bark pieces were macerated using methanol at room temperature for 48 h. Then the extracts obtained were filtered to obtain methanol extract. Thus obtained methanolic extract was concentrated in rotary flash evaporator and dried in a vacuum oven so as to obtain thick, viscous mass. The concentrated methanolic extract was subjected to successive extraction using hexane, chloroform and methanol to obtain hexane, chloroform and methanol soluble fractions. Flow-chart of overall extraction process is shown in figure 2.

Phytochemical screening

Phytochemical screening was carried out for hexane, chloroform and methanol soluble fractions as per the standard

Figure 1: Stem bark pieces of *F. auriculata*.