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In vitro antibacterial and antioxidant activities of alcoholic extract from the leaves of *Podocarpus neriifolius* D. Don

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In the present investigation, the methanol leaves extract of *Podocarpus neriifolius* D. Don was assessed for antibacterial and antioxidant activities. The antibacterial action was evaluated through agar disc diffusion method against seven pathogenic and non-pathogenic bacterial strains at various concentrations and compared against the standard (kanamycin 30 µg/disc). The antioxidant activity of the extract was investigated by means of a variety of *in vitro* assays and results were compared with standard drugs. The experiment indicates that the extract was effective against all the organisms and highest effectiveness showed against *Salmonella paratyphi* at 1000 µg/disc concentration. It also exhibited significant antioxidant activity. The total phenolic content was 168.2±6.4 mg galic acid equivalents/g of extract. The reducing power of this extract increases with the raise of concentration and the value of IC_{50} of 1,1-diphenyl-2- picrylhydrazyl (DPPH). scavenging activity was 4.73 µg/ml (IC_{50}) value of standard ascorbic acid is 5.99 µg/ml. The results provided that the studied plant might indeed be possible basis of natural antioxidant as well as antimicrobial agents.

Key words: *Podocarpus neriifolius*, antioxidant, antibacterial, 1,1-diphenyl-2- picrylhydrazyl (DPPH), reducing power, total phenol.

INTRODUCTION

Herbal medicines have been used for aid of symptoms of the disease from a long period of time (Asadbeigi et al., 2014). Traditional medicines are in trend in the remote areas of the developing and underdeveloped countries because of availability and low cost (Jakaria et al., 2015). The therapeutically important plants are frequently used in the treatment of different pathological conditions (Hasan et al., 2015). In recent years, an increased resistance development of bacterial pathogens against antibiotics has become a difficult concern caused by the

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haphazard use of up to date antibiotics (Rahman et al., 2008). Plants contains a wide diversity of secondary metabolites; these are called phytochemical constituents including tannins terpenoids, alkaloids, flavonoids etc. and some of these constituents show a broad range of *in vitro* antibacterial as well as antifungal activities (Dahanukar et al., 2000; Cowan, 2008).

Free radicals have the potential to harm cells. The gaining or losing of an electron from a molecule or atom can lead to creation of a free radical. At inferior concentration, the effect of free radicals can be minimized by body's homeostasis system but higher concentration of it can hazardously damage DNA, RNA and even cell membrane. The DNA damage may play a role in the cancer formation, ensuing in mutations that can harmfully affect the cell cycle and potentially direct to malignancy and additional fatal diseases including stroke, myocardial infarction and diabetes etc. For example, the atherosclerosis as a cardiovascular disease can be accredited to free-radical induced oxidation of numerous of the chemicals making up the body. Moreover, free radicals are responsible for alcohol-induced liver damage (Lobo et al., 2010; Ali et al., 2013). Various phyto-chemicals such as phenolic acids, flavonoids, anthocyanins, tannins and carotenoids have potent antioxidant activity and this potentiality may be used as pharmacologically active products (López et al., 2007).

Podocarpus neriifolius D. Don is a fairly large tree and medium sized to which can make up to 35 (-45) m tall. It is infrequently spurred or even buttressed while the surface of bark is grayish-brown. It is the most prevalent species of genus, geographically distributed from Nepal, India, Indo-China and Thailand, throughout malesia, towards the Solomon Island and Fiji; it is also planted in garden (Lambert, 1824; Zaman et al., 2015a). Leaves have been reported to be used as boiled water for bathing and bark decoction is applied with cotton on herpes (Zaman et al., 2015a).

In total eleven phytochemicals isolated from *P. neriifolius* were recognized as $C_{34}H_{69}OH$, β-sitosteryl stearate, β-sitosterol, sciadopitysin, podocarpusflavone B, robustaflavone-7"-methyl ether, podocarpusflavone A, robustaflavone, p-hydroxyl benzoic acid, 2"-O-rhamnosylscopariu, 2"-O-rhamnosyl vitexin (Li-zhen et al., 1993). *P. neriifolius* is reported to have antiproliferative (Shrestha, 2011), cytotoxic and thrombolytic activities. To the date, there is no scientific record concerning the antibacterial and antioxidant activities of *P. neriifolius*. So, the methanol leaves extract of the plant was assessed for antibacterial and antioxidant activities.

MATERIALS AND METHODS

Plant

For this study, fresh leaves of *P. neriifolius* were gathered from the local region of Chittagong, Bangladesh and authenticated by Professor Dr. Sheikh Bokhtear Uddin, Department of Botany, University of Chittagong, Bangladesh. The leaves were dried at room temperature for 7 days and in hot air oven for 2 days.

Preparation of extract

The dried leaves powdered and extracted with methanol for 7 with standardized shaking by using rotary shaker machine. The solvent was completely separate by filtering through No. 1 Whatman filter paper and water bath was used at 40°C to the drying of filtrates. The attained dried crude extracts were used for experiments.

Phytochemical screening

The phytochemical assessment of alcoholic extract of *P. neriifolius* was performed with the methods of Zaman et al. (2015b).

Antibacterial screening

Test organisms

Pure cultures of bacterial strains obtained from the microbiology laboratory of the Department of Pharmacy, International Islamic University Chittagong. were used as test. The crude extract was tested against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, and *Salmonella paratyphi*.

Preparation of test solution

In the case of test solution preparation, 33.33 mg of weighed crude extract was dissolved in 1 ml of distilled water then mixed thoroughly by vortex mixture. 30 µl of test solution added to each disc was corresponding to the concentration of 1000 µg/disc. Similarly, 24 and 15 µl of test solution in each disc were corresponding to 800 and 500 µg/disc respectively.

Bacterial assay

The screening for the antibacterial activity was usually performed by disc diffusion method described by Bauer et al. (1966) and Sarker et al. (2010). The extract was tested at different concentrations 500, 800 and 1000 µg/disc, the diameters of the zone of inhibition fashioned as a result of the compounds were compared with the standard antibiotic (Kanamycin, 30 µg/disc). The tests were done in triplicate.

Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of the methanol extract of *P. neriifolius* leaves was quantified according to the protocol of Ali et al. (2013). In this method, antioxidant activity was determined based on the scavenging activity of the stable free radical 1,1-diphenyl-2- picrylhydrazyl (DPPH). This activity of the crude extract was investigated at different concentrations (6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml). First of all, 2 ml crude extract was added to 3 ml of a 0.004% methanol solution of DPPH. Secondly, after 30 min absorbance was taken at 517 nm and the percentage inhibition of activity was calculated by using the following equation:

Table 1. Antibacterial activity of alcoholic extract of *P. neriifolius* leaves.

S/L code	Name of the bacteria	Zone of inhibition in mm			
		Kanamycin disc (Standard)	*P. neriifolius* leaves (methanol extract)		
		30 µg/ disc	500 µg/ disc	800 µg/ disc	1000 µg/disc
B1	*B. subtilis*	29	10.5±0.00	13.67±0.33	14.33±0.33
B2	*S. aureus*	30	10.5±0.00	12.67±33	14.33±0.67
B3	*E. coli*	32	15.67±0.33	14.33±33	14.33±0.67
B4	*P. aeruginosa*	27	11.25±0.00	15±0.58	14.67±0.33
B5	*S. typhi*	30	16.67±0.33	17.67±0.33	18.67±0.33
B6	*B. cereus*	28	16.33±0.33	16±0.58	16.33±0.33
B7	*S. paratyphi*	29	22.67±0.33	23±1.00	26.67±0.88

Values represent the average of three different values ± S.E.M.

% of the scavenging activity = $[(A_0-A_1)/A_0\times100$

Where A0 denotes the absorbance of the control and A_1 denotes the absorbance of the extract. The curves were arranged and the IC_{50} value was calculated from the graph.

Reducing power activity

The reducing power activity was evaluated according to the method described previously by Lee et al. (2009). The several concentrations of extract (62.5, 125, 250, 500 and 1000µg/ml) in 1 ml of distilled water were mixed with phosphate buffer solution (2.5 ml, 0.2 M, pH 6.6) plus potassium ferricyanide, that is, $K_3Fe(CN)_6$ (2.5 ml, 1% w/v). Then the obtained mixture was incubated at 50°C for 20 min. A fraction (2.5 ml) of 10% trichloroacetic acid was added to the mixture, which was after that centrifuged at 3000 rpm for 10 min. The upper layer of the 2.5 ml solution was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v) then the absorbance was determined at 700 nm. The increased reducing power confirmed with the increased absorbance of the reaction mixture. Ascorbic acid was used as the reference as well as phosphate buffer (pH 6.6) used as blank solution.

Total phenol content

Total phenol content of the extract was investigated by using Folin-Ciocalteu reagent according to the method of Ali et al. (2013) and Singelton et al. (1999). The sample (200 µg/ml) was mixed with 400 µl of the Folin-Ciocalteu reagent along with 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml with distilled water and then allowed to stand for 2 h. Afterward the absorbance was taken at 765 nm wavelength. The concentration of total phenol content in the extract (200 µg/ml) was then determined as mg of gallic acid equivalent by using an equation that was obtained from the standard gallic acid graph. The concentration of total phenolic compounds in sample was determined as milligram of gallic acid correspondent by using the following equation:

A = (c×v)/m

Where, A = Total phenol content (mg/ml gallic acid equivalent); c = X/1000 = Concentration of gallic acid (mg/ml); v = Quantity of extract (Volume); m = Mass of the extract (g).

RESULTS

Phytochemical screening

The phytochemical screening of methanol extract of *P. neriifolius* leaves showed the presence of terpenoids, phenols, tannins, flavonoids, alkaloids, carbohydrates, saponins, cardiac glycosides, and steroids.

Antibacterial assay

The antibacterial activity of this extract was presented in Table 1. The extract demonstrated different zones of inhibition at different concentrations (500, 800 and 1000 µg/disc) against the tested bacteria.

The extract showed the best activity at 1000 µg/disc in all the tested organisms. The maximum zone of inhibition of 26.67±0.88 mm was recorded at 1000 µg/disc concentration against *S. paratyphi*. The minimum zone of inhibition of 10.5 mm was found at 500 µg/disc concentration against *B. subtilis* and *S. aureus*.

Activity against oxidation

The DPPH radical scavenging activity

The percentage of the scavenging activity of extract and ascorbic acid at different concentration is shown in Figure 1. The value of IC_{50} tested extract as well as ascorbic acid (positive control) was found to be 4.73 and 5.99 µg/ml, respectively.

Activity of reducing power

In this test, the yellow color of the test solution transforms to different shades of green and blue depending upon the reducing power of present compounds. The potentiality of the reducing power of the extract rose with the raise in

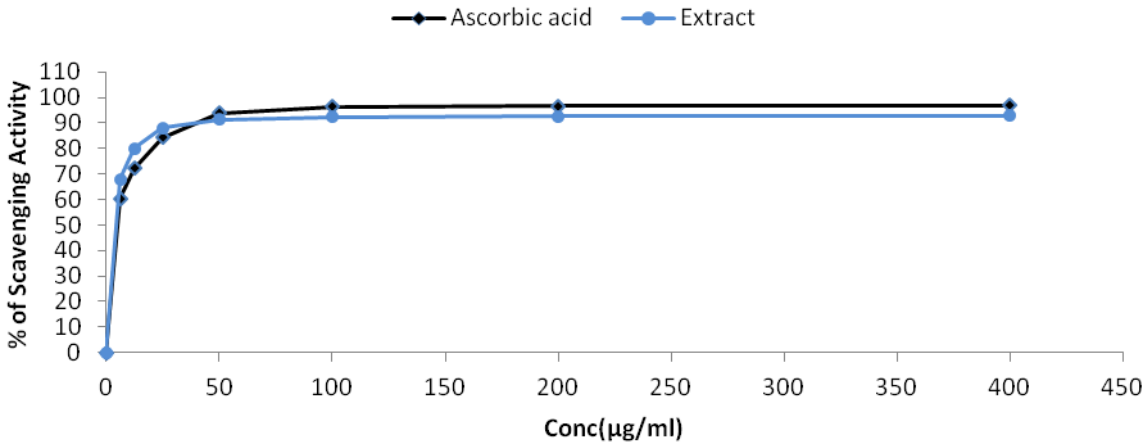

Figure 1. % of scavenging activity of the methanol leaf extract of *P. neriifolius* compared with those ascorbic acid.

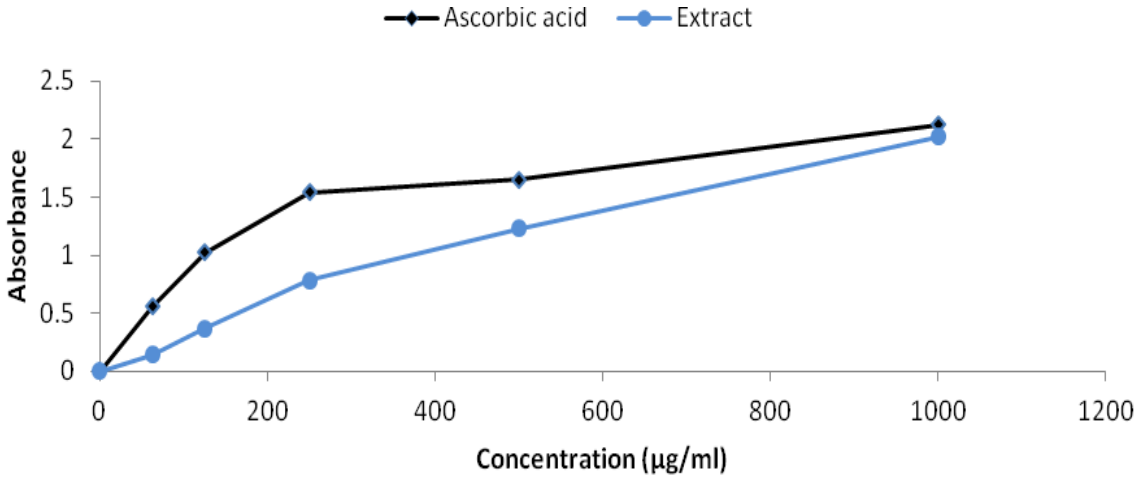

Figure 2. Reducing powers of Ascorbic acid and Methanol extracts of *P. neriifolius.*

concentration of methanol leaves extract of *P. neriifolius* and the important increasing absorbance was found to be 2.018 at 1000 µg/ml (Figure 2).

Total phenol content

The quantitative estimation of the phytochemical constituents of *P. neriifolius* shows that the medicinal plant is rich in total phenols. The estimated total phenol was 168.2±6.4 mg GAE/g extract.

DISCUSSION

The study aimed to inspect the antibacterial and antioxidant activities of alcoholic extract from the leaves of *P. neriifolius*.

The investigations of the extract aimed to find the active phytochemical constituents present in this extract and valuable compounds founded in this extract. Then, antibacterial activity was assessed against. According to the result of antibacterial activity tests, this extract inhibits the growth of bacteria. Among the seven bacteria, this extract produced greatest inhibition against *S. paratyphi* and *S. typhi*. Literature revealed that, herbal products from the natural resources represent a promising source of antimicrobial agents for the reason that they are natural and affordable, particularly for rural communities (Ghosh et al., 2008). Approval of medicines from herbal sources as another form of medical care is increasing since they are serving as sources of novel antibiotic prototypes. It has been reported that various phyto-chemical active compounds including glycoside, saponin, tannin, flavonoids, terpenoid, and alkaloids are responsible for antimicrobial activity in various plant species

(Ebi and Ofoefule, 1997; Devi et al., 2012; Itoandon et al., 2012). This study suggests that, *P. neriifolius* possesses antibacterial activity because of these phytochemical constituents.

The investigation of antioxidant activity and this activity was also found in this extract. *In vitro* antioxidant tests permit quick estimation and identification of compounds as antioxidant agents because the substances have low antioxidant (Saeed et al., 2012). Regarding the results of the DPPH radical scavenging activity it is recommended that the plant extract contain phytochemical constituents that were capable of donating hydrogen to a free radical to scavenge the probable harm. The presence of redactors has reducing activity of a compound, which showed antioxidative property probable by breaking the free radical chain, donating a nitrogen atom. The attendance of reductants in *P. neriifolius* extracts results in the reduction of the Fe^{2+} ferric cyanide compound to the ferrous form. The phenolic compounds from the plants were also very significant because their hydroxyl groups confer scavenging ability. From the results of total phenolic content test, this extract was affluent of total phenol also responsible for antioxidant activity.

This research study suggests that the methanol extract of *P. neriifolius* leaves possesses antibacterial and antioxidant activity. But through which mechanism the extract inhibits the bacterial growth is unknown. Additional studies would be essential to estimate the involvement of active chemical constituents for the experimental antibacterial activity as it still remains to be determined which compounds were accountable for these effects. In near future this plant might be a contributor in the field of allopathy and/or naturopathy as a potent therapeutically active drug.

Conflict of Interests

The authors have not declared any conflict of interests.

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