

Antioxidant and antimicrobial activity of Ethanolic extract of *Ophiorrhiza brunonis* (Rubiaceae)

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Abstract

Antioxidant and antimicrobial potential of ethanolic extract of *Ophiorrhiza brunonis* leaf was investigated by analysing Reducing power assay, Hydrogen peroxide scavenging assay and Nitrate reductase assay using Ascorbic acid as standard. The extract was subjected to antimicrobial activity against two gram-positive bacteria (*Bacillus cereus* and *Enterococcus faecalis*), two gram-negative bacteria (*Escherichia coli* and *Proteus mirabilis*) and two fungal strains (*Aspergillus niger* and *Rhizopus stolonifer*), which cause food borne illness and infections. According to the findings revealed significant antioxidant activity (IC₅₀ 80 µg/ml). A positive linear regression was observed between the extract and the standard. Therefore *O. brunonis* having regression (R²) values for reducing power assay, Hydrogen peroxide scavenging assay and nitrate reductase scavenging assay with 0.993, 0.961 and 0.981 respectively. The ethanolic extract of *Ophiorrhiza brunonis* showed inhibitory activity against *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli* and *Aspergillus niger* with mean zones of inhibition of 10.93, 13.3, 14.23 and 11.5mm respectively. The present study showed that the extracts of *Ophiorrhiza brunonis* might be a potential candidate to serve as natural antioxidant and antibiotic agent.

Introduction

Infectious disease caused by bacteria and fungi is a major threat to the mankind as well as to the animals. There are number of antibiotics available in the market but the issue is not with antibiotics available commercially, but the problem starts by the generation of antibiotic resistant microorganisms. It is tedious to develop new drugs with novel technologies (Anjana Sharma *et al.*, 2009) so the science community is rushing to discover new drugs in order to combat such diseases (Abeyasinghe 2010). The developing nations believe on plant based medicines for treating many diseases. In industrialized countries about 30% of the drugs prescribed are from plant origin (Olusola Adeyanju *et al.*, 2011). In contrast to chemical or synthetic drugs antimicrobials of plant origin have reduced side effects (Piddock and Wise, 1989). So majority of the world population in developing countries prefers plant based drugs. The present study focus on *Ophiorrhiza brunonis* an endemic plant to Western Ghats belongs to the family Rubiaceae. The genus *Ophiorrhiza* (L) is distributed throughout the Indo-Malesian region and has its greatest diversity in south east Asia and New Guinea (Mabberley, 2005). In India the plant is distributed in the Western Ghats with 42 species (Deb and Mondal, 1997). The genus name was derived from greek origin “Ophios” refers (a snake) “rhiza” refers to root. The root is used to treat snake bite.

Materials and Methods

Plant material: *Ophiorrhiza brunonis* was collected from Megmalai Wildlife Sanctuary of Theni District, Western Ghats and the voucher specimen was deposited in the Sri Ganesan Herbarium (SGH), Department of Botany, The Madura College, Madurai, India.

Preparation of Plant extract

The plant sample was shade dried and powdered using an electric grinder. The sample was subjected to extract with ethanol using Soxhlet's apparatus for 6 hours continuous reflux and the ethanol extract was used for further studies.

Assessment of Antioxidant activity

Reducing power assay

Reducing power was determined by the method prescribed by (Jafri *et al.*, 2014). About 100 µl of plant extract at various concentrations was mixed with 500 µl of 2M phosphate buffer (pH 6.6) and 500 µl of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. After incubation, 500 µl of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the mixture (500 µl) was mixed with an equal volume of distilled water and 100 µl of 0.1% ferric chloride solution. The absorbance was measured at 700 nm. The reducing power of the sample was calculated using the formula

$$\text{Reducing power \%} = \frac{A_{\text{Control at 700nm}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay was carried out by following the procedure of (Jayaprakasha *et al.*, 2004). 100 µl of plant sample was added in 2 ml of 20mM hydrogen peroxide solution prepared in phosphate buffer saline (pH 7.4). After 10 min, the absorbance was measured at 230 nm against a blank solution that contained hydrogen peroxide solution without the extract. The percentage of H₂O₂ scavenging of the extract was calculated as follows: % Scavenged [H₂O₂] = [(Abs control – Abs sample) / Abs control] × 100.

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside was measured according to the method of (Marcocci *et al.*, 1994). Briefly, 5 ml of reaction mixture containing 5mM sodium nitroprusside in phosphate buffered saline (pH 7.3), with or without the plant extract at different concentrations was incubated at 25°C for 180 min in front of a visible polychromatic light source. The Nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion which was assayed at 30 min intervals by mixing 1 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylene-

diaminedihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylene- diaminedihydrochloride was measured at 546 nm.

Antimicrobial activity

Microorganism's used for the study: Antimicrobial activity of the plant extract was determined by well diffusion method. In this study, four bacterial strains namely (*Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*) and two fungal strains (namely *Aspergillus niger*, and *Rhizopus stolonifer*) were used. The media used for antibacterial test were nutrient agar. The fungal strains cultured on Potato dextrose agar (PDA) for susceptibility tests. Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50µl of plant extract by using micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30minutes. Further the plates were incubated in an incubator at 37°C for 24hours and 28-30°C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

Results and Discussion

Antioxidant assays

The plant extract showed significant antioxidant activity and the results were shown in (Fig. 1-3). The test extract showed good activity compared to the standard drugs. Among the three assay tested, nitrate reductase scavenging assay-showed significant result.

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power is associated with the presence of reductones (Kumaran and Karunaka, 2007). There was a dose dependent increase in the percentage of antioxidant activity for all concentrations tested and the same in different test opted for antioxidant assay such as hydrogen peroxide scavenging assay and nitrate reductase scavenging assay. The IC_{50} for all the three test were found in the range of 80 µg/ml. The

scavenging activities of all the assays were significant when compared to the standard (Ascorbic acid). A positive linear regression was seen between the extract and the standard having a regression (R^2) values of 0.993, 0.961 and 0.981 respectively.

Fig:1 Reducing power assay of ethanol extract of *Ophiorrhiza brunonis*

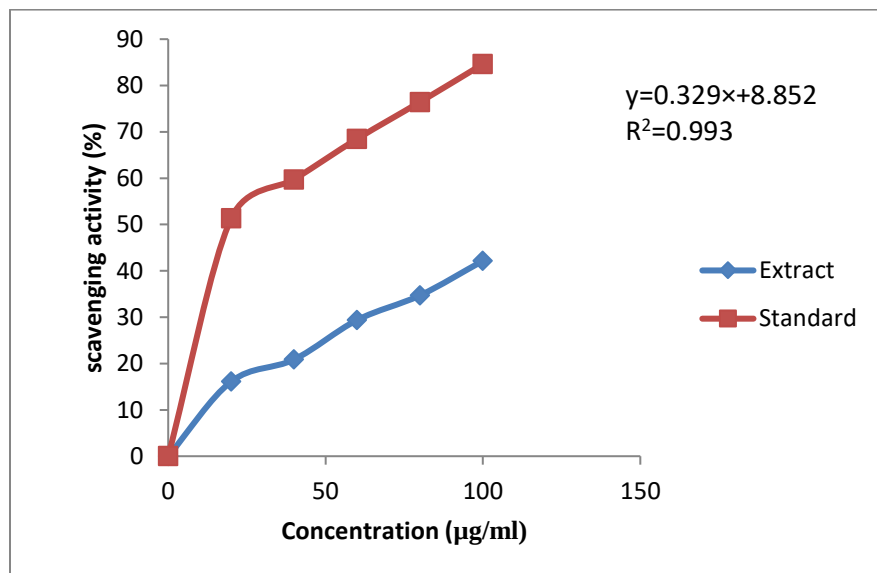


Fig:2 Hydrogen peroxide scavenging assay of ethanol extract of *Ophiorrhiza brunonis*

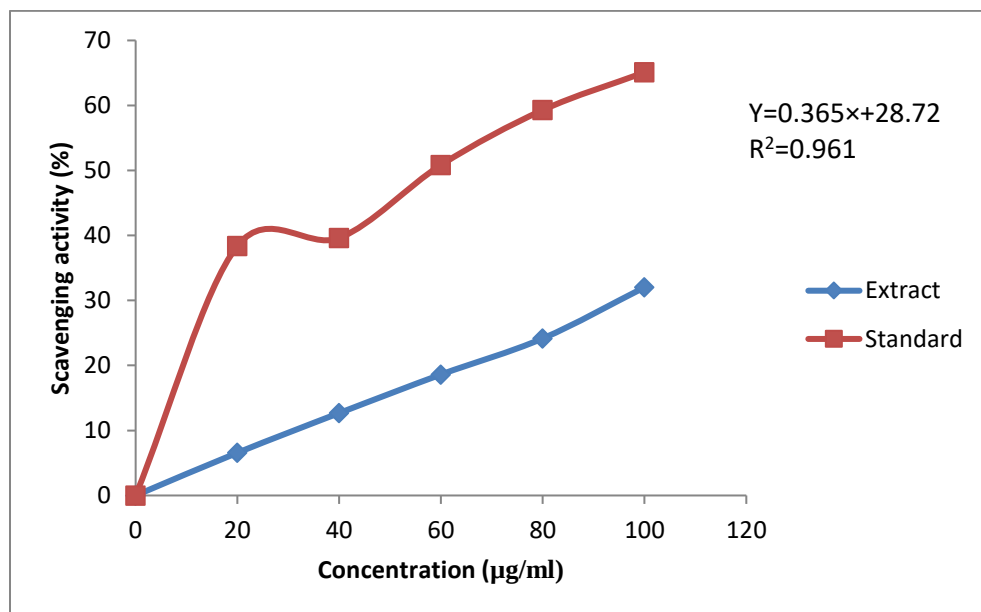
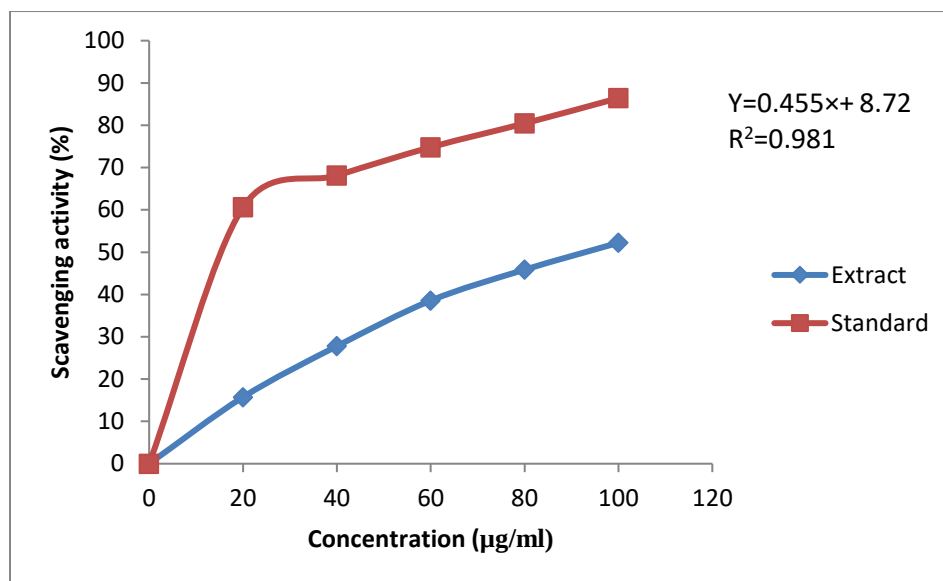


Fig:3 Nitrate reductase scavenging assay of ethanol extract of *Ophiorrhiza brunonis*



Antimicrobial activity

The ethanolic extract of *Ophiorrhiza brunonis* was tested against two Gram positive bacteria (*Bacillus cerus*, *Enterococcus faecalis*), two Gram negative (*Escherichia coli*, *Proteus mirabilis*) and fungi (*Aspergillus niger*, *Rhizopus stolonifer*). According to the results shown in (Table 1) the extract exhibited potent inhibitory effect against the selected organisms with diameter of inhibition zones ranging from 10.93mm to 14.23mm. The extract showed high antibacterial effect on *Escherichia coli* and *Enterococcus faecalis* with the diameter of inhibition zone of 14.23, 13.3mm and it is moderately active on *Bacillus cerus* with the zone of inhibition 10.93mm. The extract exhibited good antifungal activity on *Aspergillus niger* with a maximum zone of inhibition of 11.5mm (Table 2). Whereas, in *Proteus mirabilis* and *Rhizopus stolonifer* were not susceptible to the plant extract.

Table: 1 Antibacterial activity of ethanol extract of *Ophiorrhiza brunonis*

Name of Organism	Zone of Inhibition (mm)		
	Test	+ ve control (Erythromycin)	-ve control
<i>Bacillus cereus</i>	10.93 ± 0.40	15	NA
<i>Enterococcus faecalis</i>	13.3± 0.3	24	NA
<i>Escherichia coli</i>	14.23 ±0.25	15	NA
<i>Proteus mirabilis</i>	NA	NA	NA

NA- No activity

Table: 2 Antifungal activity of ethanol extract of *Ophiorrhiza brunonis*

Name of Organism	Zone of Inhibition (mm)		
	Test	+ ve control (Clotrimazole)	-ve control
<i>Aspergillus niger</i>	11.5 ± 0.45	16	NA
<i>Rhizopus stolonifer</i>	NA	21	NA

NA- No activity

Antioxidant scavenging activity of ethanolic extract of *O. brunonis* expressed remarkable activity. The result of reducing power, hydrogen peroxide scavenging assay and nitrate reductase scavenging assays are dose dependent. Reducing power increases with an increase in the absorbance of extract and standard at 700nm as reported in *O. protesta* and *O. mungos* (Krishnakumar *et al.*, 2012) as the concentration increases activity also increases. The present results are also comparable with above investigations. The scavenging activities are significant when compared with the standard drugs used. Similar results observed with positive correlation coefficient in *O. mungos* (Farhana *et al.*, 2011). The hydrogen peroxide scavenging assay also exhibited a dose dependent activity. Hydrogen peroxide plays an important role in the transformation of malignancy, but it can also sensitize cancer cells to H₂O₂ induced cell death (Miguel Lo'pez-La'zaro 2007).

Bioactive compounds present in the extract inhibited the growth of microorganisms by exhibiting zone of inhibition. In the present study highest activity was found in *E. Coli*. It was

found that maximum zone of inhibition against *E. coli* was reported in *Galium* (Rubiaceae) species (Filiz *et al* 2017), *O. trichocarpon* Bl.var. trichocarpon, *O. rugosa* and *O. aff.nutans* Cl.ex Hk. f. (Nichakan Phoowiang *et al.*, 2009). Concurrently it was observed that *E. coli* showed no activity in *O. Mungos* (Jayadev *et al.*, 2013). Flavanoids are bioactive compound inhibit the growth of *Bacillus cerus* (Hidetoshi Arima *et al.*, 2002). Reports suggest that *E.coli* is responsible for bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea (Tarun Madappa 2017). *Proteus mirabilis* and *Rhizopus stolonifer* does not showed any activity against the plant extract it does not mean that the plant has no active compounds to inhibit the growth of such organisms. Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria (Rameshkumar *et al.*, 2007; Stefanello *et al.*, 2008; Tajkarimi *et al.*, 2010). It may be effective on altering the concentration of the extract. But in gram positive bacteria there is a mesh like peptidoglycan layer which is more accessible to permeation by the extracts (Burt, 2004; Qa'dan *et al.*, 2005; Rameshkumar *et al.*, 2007; Stefanello *et al.*, 2008).

Conclusion

The ethanolic extract of *Ophiorrhiza brunonis* exhibited by significant antioxidant and antimicrobial activity. The extract was subjected to antimicrobial activities with Gram (+), Gram (-) and selected fungi. Among these microorganisms, the maximum antibacterial activity is found in Gram negative bacteria *Escherichia coli* and potent antifungal activity in *Aspergillus niger*.

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