ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF PHYLLANTHUS EMBLICA FOR ITS APPLICATION IN TREATMENT OF OPHTHALMIC DISORDERS

S.S.SAWHNEY*, R.M.PAINULI**, DOLLY SINGH**

* Uttarakhand College of Science and Technology, Dehradun, India. **H.N.B. Garhwal Central University, Srinagar, Garhwal, India.

ABSTRACT

The oxidation stress is known to be a reason for various tissue degeneration and plays a role in premature aging. In wake of this, there is need to explore a natural antioxidant which can help body maintain a balance between inbuilt oxidation stress and detoxifying reactions. We present the antioxidant power of various extracts of Phyllanthus emblica which was tested by FRAP method. The phytochemical analysis of the extracts was checked by HPLC. Along with the standard compound, gallic acid, the peaks were analyzed. The peaks showed the compound present was mainly, Gallic acid. The antioxidant activity was found to be maximum in the order of Ethyl Acetate extract followed by Acetone, Methanol and Aqueous extract. Along with antioxidant activity the extracts were tested as an anti-microbial agent for the pathogens known to cause various eye infections. The minimal inhibitory concentration was calculated and was found to be 1mg/ml for S.mutans, S.aureus and S.pyogenes and 0.6mg/ml for S.pneumoniae. Phyllanthus emblica is a powerful antioxidant along with its activity to inhibit the growth of the pathogens makes us believe that it can be used as new drug compound for preparation of eye drops and also in various therapeutic.

Keywords: Phyllanthus emblica, HPLC, Antioxidant, Eye infection, Broad-spectrum antibiotic, MIC

INTRODUCTION

Oxidative Stress (OS) is a generally used to describe the sturdy state of oxidative damage in a cell or tissue and can also effect the normal function of organs which is caused by the reactive oxygen species (ROS). This oxidative stress affects either a specific molecule or the organism as a whole. Reactive oxygen species represent a class of molecules that are derived from the metabolism of oxygen and are inherently found in all aerobic organisms in form of free radicals and peroxides. Energy-derived metabolic activity or endogenous source are the primary source of production of such free radicals such as molecules that are derived from the metabolism of oxygen and are inherently found in all aerobic organisms in form of free radicals and peroxides. Energy‐derived metabolic activity or endogenous source

Present day clinical treatments are having a setback because of the ever-evolving nature of microorganisms, which have developed resistance to them, and hence treatment of infectious diseases is problematic scenario. The infections caused by bacteria, viruses and fungus can also lead to the build up of the reactive oxygen species in the body. Oxidative damage is majorly observed in the eyes particularly affecting the retina and the lens, is an also known contributing factor to age-related ocular degeneration and cataract. The present study in detail describes the extraction procedure, phytochemical analysis, antioxidant power and screening of the antimicrobial activity of Phyllanthus emblica against pathogens especially known to cause various eye infections.

MATERIAL AND METHODS

Materials

The plants material (seeds) was collected from the local market in Mumbai. Mueller-Hinton agar was purchased from Hi-Media, Mumbai. Bacterial Inoculum was procured from IMTECH chandigarh, India. Chemicals used for HPLC analysis were of HPLC grade. The microorganisms used in this study are Streptococcus mutans, Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa. These microorganisms were procured from the IMTECH, Chandigarh, INDIA.

Preparation of plant extracts

Dried fruits were powdered in the grinder and stored in the airtight container in the dark until further use. 25g of the powder of seeds of P.emblica were taken in a thimble and the extracts were prepared in the series of 500ml of different solvents based on increasing polarity (Ethyl acetate, Acetone, Methanol, and Water) using Soxhlet extraction method. The solvents were evaporated to dryness using rotary evaporator and the extracts were lyophilized at ~50°C. The lyophilized extracts powder was stored in airtight bottles at 4°C till further experimentation.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was carried out using C18 PCX 500 Dionex analytical column, (WATER 2414 refractive index detector and 515 HPLC pump) with 0.1 M KCl, 0.05 M HCl and 10% acetonitrile as the mobile phase. The detection was carried out at 260nm using UV detector. Peak areas were identified, and gallic acid was used as an external standard.
Antioxidant Power by FRAP Method

The Ferric-Reducing Antioxidant Power (FRAP) assay measures the antioxidant potentials of "antioxidants" to reduce the Fe3+/2,4,6-tripyridyl-s-triazine (TPTZ) complex present in a stoichiometric excess to the blue coloured Fe2+ form. The stock solution of various extracts of concentration 0.3mg/ml was prepared. The tubes were incubated at 37°C for 15minutes. The absorbance was read at 593nm in spectrophotometer, which was set zero with blank (FRAP reagent and distilled water).

The experiment was repeated in triplicates and represented as mean value average ± S.D.

Table 1: Gives the composition of the test solutions for FRAP

<table>
<thead>
<tr>
<th>Concentration</th>
<th>FRAP Working Solution (ml)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6x10^-4</td>
<td>1.5</td>
<td>3.49</td>
</tr>
<tr>
<td>12x10^-4</td>
<td>1.5</td>
<td>3.48</td>
</tr>
<tr>
<td>18x10^-4</td>
<td>1.5</td>
<td>3.47</td>
</tr>
<tr>
<td>24x10^-4</td>
<td>1.5</td>
<td>3.46</td>
</tr>
<tr>
<td>30x10^-4</td>
<td>1.5</td>
<td>3.45</td>
</tr>
<tr>
<td>36x10^-4</td>
<td>1.5</td>
<td>3.44</td>
</tr>
<tr>
<td>42x10^-4</td>
<td>1.5</td>
<td>3.43</td>
</tr>
<tr>
<td>48x10^-4</td>
<td>1.5</td>
<td>3.42</td>
</tr>
<tr>
<td>54x10^-4</td>
<td>1.5</td>
<td>3.41</td>
</tr>
<tr>
<td>60x10^-4</td>
<td>1.5</td>
<td>3.40</td>
</tr>
</tbody>
</table>

Determination of antimicrobial activity

For antibacterial activity study, Ethyl acetate, Acetone, Methanol, and aqueous extracts were dissolved in DMSO to a final concentration of 10mg/100μl.

Susceptibility test by well diffusion method

The inoculum size of the test strain was standardized according to the National Committee for Clinical Laboratory Standards. The test bacterial strains were inoculated into Mueller Hinton Broth medium and incubated for 24hrs. The plates were incubated at 37ºC was 15minutes. The least concentration of the extract or standard drug showing no visible growth was taken as the MIC. The results were tabulated after 24hrs of incubation period and its mean MIC value was calculated.

RESULTS AND DISCUSSION

Phytochemical analysis of Plant Extract using HPLC

Plants are essential source of potent compounds for the development of new drug moieties. The major step for this is examining the properties of these plants for being an antibacterial and antioxidant agent. Various groups have evaluated the antibacterial, antifungal, antiviral, and anti-inflammatory properties of plants. Some of these findings have helped in identifying an active principle compounds that are responsible for such activities and in the developing drugs for the therapeutic use.

The HPLC analysis of the various extracts was carried out to identify the phytochemicals, responsible for its antioxidant and antimicrobial activity. Gallic acid was identified as the major compound in Ethyl acetate, Acetone extracts. Where as, in Methanol though Gallic acid was detected to be a major compound, but the presence of other types of compounds has led to enhance the antimicrobial activity though showing variation in a antioxidant property of the extract. In aqueous extract the amount percentage of gallic acid is very less. The HPLC analysis of various extracts shows various peaks with retention times of 6.584, 6.579, 6.667, and 6.447, which corresponded to the retention time of Gallic acid chosen as a standard compound.

Antioxidant activity of Phyllanthus emblica

Free radicals are believed to be involved in major disorders like neurodegenerative diseases, muscular dystropin, AIDS etc. Antioxidants agents through their scavenging power are valuable in the management of many such diseases. The Ferric-Reducing Antioxidant Power (FRAP) assay measures the antioxidant potentials of “antioxidants” to reduce the Fe3+/2,4,6-tripyridyl-s-triazine (TPTZ) complex present in a stoichiometric excess to the blue coloured Fe2+ form. The free radical scavenging activity in the different plant extracts decreased in the following order: Ethyl acetate > Acetone > Methanol > Aqueous. All the extracts of P.emblica at different concentrations exhibited more than 70% scavenging activity (Fig 1-4). This study suggests that the plant extract retained its antioxidant activities during the course of extraction and can counteract the oxidative damage induced by the pathogens responsible for various ophthalmic disorders. These extracts can be designed as drug moiety in treatment of severe disorders.
Susceptibility of microbes to *P. emblica*

The microbes chosen in this study were found to be involved in different ophthalmological infections. The results (Table 2 and 3) show all the extract of *P. emblica* having strong inhibiting power on the growth of microorganism. The aqueous extract was found to less effective when compared to other extracts since the average inhibition zone diameter was found to be ranging from 5.33mm to 8mm which was lesser than other three extracts which showed the IZD value to be ranging from 6.67mm to 12mm for various pathogens in study. The extraction procedure does not seem to have any affect in antimicrobial activity.

The crude and extracted form was both found to have high antimicrobial activity. The zone of inhibition ranging more than
CONCLUSION

The result of the present study shows that the extract of *P. emblica*, which contain highest amount of phenols, tannins, lignins, flavonoids (as reported in early works), possessed the highest antioxidant activity. The scavenging property of extract of *P. emblica* can be due to hydroxyl groups present in the phenolic compounds in the plant extract and this chemical structure helps in providing the necessary component as radical scavenger. Free radicals are often resultant product of biological reactions or due to exogenous factors. Free radicals involvement in the various pathogenesis is well documented and it is also well established that a potent scavenger of free radicals can serve as a possible intercession for the diseases. All of the extracts of *P. emblica* in this study exhibited different range of antioxidant activity along with microbial inhibition. The microbes screened are mostly involved in eye related disorders, *S. aureus* and *S. pneumoniae* are the causative agent of Keratitis and Iritis, *S. pneumoniae* and *S.pyogenes* causes septicemia and postoperative traumatic. All these pathogens are all responsible for periorbital and orbital cellulites. The susceptibility of these pathogens at low concentration of extract opens up arena of using *P. emblica* as new drug compound for treatment of various ophthalmological disorders.

REFERENCES