Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Applied Sciences
1(1), 2013, 42-49

ISSN 2321 – 7456

Original Research Article

ASSESSMENT OF ANTIMICROBIAL AND ANTI-OXIDANT ACTIVITY OF MORINGA OLEIFERA LEAVES.

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Abstract:

Background: Various complex chemical constituent presence in plant which are found in secondary metabolite as alkaloids glycosides carticosteroids, essential oil etc. the use of active chemical constituent is obvious since their compounds are having a fixed definite pharmacological effect. The activity of plant against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compound in the plant. Many herbal plant contain antioxidant compounds protect cells against the damaging effects of reactive oxygen species. ***Objectives:*** In the present study an attempt was made to carryout the anti-microbial activity of leaves *Moringa Oleifera* against organisms like *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa* and antioxidant activity. ***Methodology:*** The activity of all five extracts and Pet. ether precipitate were evaluated by disc diffusion method. This method is based on diffusion of antibacterial component from filter paper disc to the surrounding inoculated nutrient agar medium, so that the growth of microorganisms is inhibited as circular zone around the disc. Two gram positive and two gram negative organisms were selected viz. Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis for the present study. The antioxidant activity of Plant extract was determined by different in-vitro method such as, the DPPH free radical scavenging assay method. The extract was dissolved in DMSO at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered. ***Result:*** Highest zones of inhibition were found in methanolic extract of powdered leaves against all the bacteria tested. The methanol extract possess significant antioxidant activity compared to standard ascorbic acid. ***Conclusion:*** The consequence of this investigation suggest that the extract can be used to discover antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria responsible for severe illness.

Keywords: *Moringa oleifera, Pathogenic bacteria,* Anti- microbial and antioxidant

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Received on: April 2013
Accepted after revision: June 2013
Downloaded from: www.johronline.com

1. Introduction:

A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana proposed for their interesting antioxidant activities [1]. Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite [2], [3]. When the balance between ROS production and antioxidant defenses is lost, Oxidative stress results which through a series of events deregulates the cellular functions and leads to various pathological conditions including aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neurodegenerative diseases [4]. Antioxidants can neutralize the ill effects of free radicals by scavenging or chain breaking (like vitamin A, C, beta carotene, etc.) or some other mechanism of action. These antioxidants must be constantly replenished since they are used up in the process of neutralizing free radicals [5]. The antioxidative potential of phenolic compounds can be attributed to their strong capability to transfer electron to ROS/free radicals, chelating metal ions and to activate antioxidant enzymes [6]. DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age related development of the major cancers [7], [8]. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have restricted use in food industry as they are suspected to be carcinogenic [9]. Therefore, the antioxidants with natural origin have been the centre of attraction for the modern researchers.

The benefits for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of Moringa preparations (e.g. extracts, decoctions, poultices, creams, oils, emollients, salves, powders, porridges) are not quite so well known [10]. Although the oral history here is also voluminous, it has been subject to much less intense scientific scrutiny, and it is useful to review the claims that have been made and to assess the quality of evidence available for the more well-documented claims. The readers of this review are encouraged to examine two recent papers that do an excellent job of contrasting the dilemma of balancing evidence from complementary and alternative medicine (e.g. traditional medicine, tribal lore, oral histories and anecdotes) with the burden of proof required in order to make sound scientific judgments on the efficacy of these traditional cures [11], [12]. In many cases, published *in-vitro* (cultured cells) and *in-vivo* (animal) trials do provide a degree of mechanistic support for some of the claims that have sprung from the traditional medicine lore. For example, numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with Moringa or with phytochemicals isolated from Moringa [13], [14]. [15], [16]. In the present study an attempt was made to carryout the anti-microbial activity of the leaves of *Moringa Oleifera* against organisms like *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and antioxidant activity.

2. Materials and Method:

2.1. Plant collection and Authentification

Dried leaves of *Moringa oleifera* plant is collected from the local area of Bilaspur, Inda and dried *leaves of Moringa* oleifera was authenticated by Dr.Shiddamallaya N, National

Ayurveda Dietetics Research Institute Bangalore-560011 (RRCBI/MCW/06).

2.2 Method of extraction [17]

The method is based on the extraction of active constituents present in the drug, using various solvents ranging from non-polar to polar. The solvents used were petroleum ether, benzene, chloroform, methanol and water. The extraction was done using soxhlet apparatus. The extraction vessel was made up of borosil glass which contain round bottomed flask i the plant material to be extracted was packed in the soxhlet assembly and a condenser through which refluxing was done. Heat was supplied through a heating mantle. The extract was collected directly from round bottomed flask and solvent was evaporated using rota evaporator.

3. Anti microbial screening of *Moringa oleifera* plant extracts. [18]

The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms.

3.1 Preliminary Antibacterial Screening

The activity of all five extracts and Pet. ether precipitate were evaluated by disc diffusion method. This method is based on diffusion of antibacterial component from filter paper disc to the surrounding inoculated nutrient agar medium, so that the growth of microorganisms is inhibited as circular zone around the disc. Two gram positive and two gram negative organisms were selected viz. Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis for the present study.

3.2 Organism used

Standard cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis* were obtained from Microbiology Department of Govt.Science College, Bilaspur Staining techniques and biochemical reactions identified the microorganisms. The organisms were maintained by sub-culturing at regular intervals in nutrient agar medium.

3.3 Preparation of extracts

Plant extracts were prepared by successive Soxhlet extraction method. The plant materials (leaves and stem) were powdered and extracted successively with different solvents (non-polar to polar). The plant extracts were filtered through Whatman filter paper into beaker. The filtrates were dried until a constant dry weight of each extracts was obtained. The residues were stored at 4°C for further use.

3.4 Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrien agar broth for bacteria that were incubated without agitation for 24 hrs at 37°C.

3.5 Antimicrobial susceptibility test

The disc diffusion method was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using nutrient agar media obtained from Himedia (Mumbai). The nutrient agar plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts were loaded on 3mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate by using standard drugs (30µg/disc Penicillin for bacteria).

4. Anti Oxidant Activity:

Antioxidant activity of different extracts was tested by the DPPH assay method.

4.1 Anti Oxidant Activity:

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. The free radical DPPH is

purple in color in methanol and is reduced to the corresponding hydrazine, which is yellow in color, when it reacts with hydrogen donor. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in the absorbance is measured at 490 nm. Preliminary qualitative analysis [19], [20] showed the presence of flavonoids, tannins, steroids, proteins and starch. The methanol extract was used for antioxidant studies.

4.2 Antioxidant assay

The antioxidant activity of Plant extract was determined by different in-vitro method such as, the DPPH free radical scavenging assay method [21]. The extract was dissolved in DMSO at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered.

4.3 Preparation of test and standard solution

The extract and standard, ascorbic acid (2mg each) were separately dissolved in 5ml of DMSO. The solutions were serially diluted with freshly distilled DMSO to obtain the lower dilutions.

4.4 DPPH Radical scavenging activity

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH [22], [23] 0.1 mm solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (50-250µg/ml). It was incubated at 37 ^{o}C for 30 minutes and the absorbance was measured at 517 nm UV-Visible Spectrophotometer (Systronics UV-Visible Spectophotometer 117, INDIA).against the corresponding blank solution. The assay was performed in triplicate. Ascorbic acid was taken as standard. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contain DPPH and distilled water. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

5. Results:

5.1 Zone of inhibition of different extracts

According to present study preparing extracts with organic solvent was shown to provide a better antibacterial activity (Table no.1 & Fig 1).

5.2 DPPH radical scavenging activity or Anti oxidant activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity. The percentage of DPPH racial scavenging activity presented in (Table. 2). The DPPH radical scavenging activity increases with increasing concentration of methanol extract of *Moringa oleifera*. 250 µg/ml of TCM and 10 µg/ml ascorbic acid (std.) exhibited 75.67% and 93.33% inhibition, respectively. The inhibitory concentration at 50% was found to be 155.81 µg/ml and 6.4 µg/ml for TCM and ascorbic acid (std.) respectively. The results indicate that the methanol extract possess significant antioxidant activity compared to standard ascorbic acid.

Table no 1: Zone of inhibition of different extracts

Organisms	*Zone of inhibition of extracts in mm*					*STD*
	P.E	**B.E**	**C.E**	**Me.E**	**Aq.E**	
1. *Staphylococcus*	7	7	6	8	NI	10
2. *Pseudomonas*	7	6	6	8	NI	10
3. *Bacillus*	6	5	6	7	NI	10
4. *E. coli*	7	6	6	8	NI	10

NI=No Inhibition **P.E**= Petroleum ether extract, **B.E**= Benzene extract**, C.E**= Chloroform extract**,**

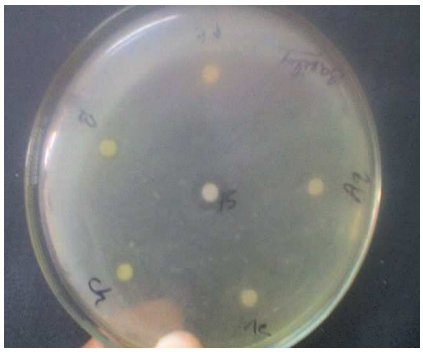
Me.E= Methanolic extract**, Aq.E**= Aqueous extract **STD**=Standard (Penicillin 30µg/disc)

Table No.2: Data shows % of antioxidant activity of methanol extract

Standard			**Sample**		
Concentration	**absorbance at 517nm**	**%of activity**	**Concentration**	**Absorbance at 517nm**	**Concentration**
2 µg/ml	0.673	50µg/ml	39.36	0.870	21.62
4µg/ml	0.479	100µg/ml	56.84	0.672	39.45
6µg/ml	0.282	150µg/ml	74.59	0.478	56.93
8µg/ml	0.159	200µg/ml	85.67	0.355	68.01
10µg/ml	0.074	250µg/ml	93.33	0.270	75.67

Figure No.1 Zone of inhibition of different extracts

Fig a **Fig b**



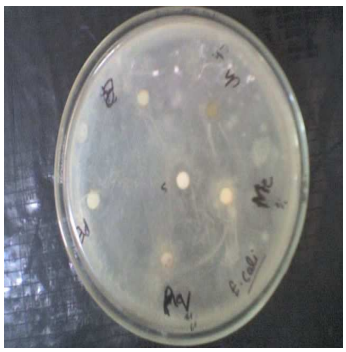


Fig c **Fig d**

PLATES:

- a – Bacillus.
- b – E.coli.
- c – Pseudomonas.
- d – Staphylococcus.

DISCS:

P E – Pet ether extract.
Me – Methanolic extract.
Ch – Chloroform extract.
B – Benzene extract.
Aq– Aqueous extract
St – Standard Penicillin

6. Discussion:

The leaves of *moringa oleifera* belonging to family *Moringaceae* was selected for my project on the basis of ethno botanical information which reveals its uses against micro organism. Literature Survey showed that very less work has been performed on this plant and ethno botanical information about the uses of the leaf part of the plant showing activities against various different both gram positive and gram negative bacterias. So, we planned to work on this project and select disc diffusion method for antimicrobial screening. Successive solvent method was used for extraction of powdered leaves .In another side we aslo performed antioxidant activity of methanol extract of powdered dried leaves of moringa oleifera. Antioxidant activity of different extract was tested by the DPPH method.

We studied antimicrobial activity because of today, most pathogenic organism are becoming resistant to antibiotics [24]. *Moringa oleifera* was suitable plant for this present study since moringa oleifera leaves contain bio component whose antibacterial potential may be highly comparable with that of the antibiotic penicillin against gram positive and gram negative

bacteria tested. Moringa leaves have been reported to be a good source of natural antioxidants such as ascorbic acid, avo-noids, phenolics, and carotenoids [25]. So another study was antioxidant activity on methanolic extract of *moringa oleifera* leaves. In accordance with the result obtain in investigation, zones of inhibition were found in pet. Ether, benzene, chloroforms and methanolic extracts against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.* Aqueous extract did not show inhibition zone against all tested bacteria (Table 1). The DPPH radical scavenging activity increases with increasing concentration of methanol extract of *Moringa oleifera*. 250 µg/ml of TCM and 10 µg/ml ascorbic acid (std.) exhibited 75.67% and 93.33% inhibition, respectively. The inhibitory concentration at 50% was found to be 155.81µg/ml and 6.4µg/ml for TCM and ascorbic acid (std.) respectively (Table 2). The results indicate that the methanol extract possess significant antioxidant activity compared to standard ascorbic acid.

7. Conclusion:

According to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity. Highest zones of inhibition were found in methanolic extract of powdered leaves against all the bacteria tested which was only 15-20% lowers effective in respect of known slandered drug. Methanolic extract exhibited 75.67% and 93.33% inhibition; respectively. That means sample posses significant antioxidant activity as compared to standered drug. To overcome the alarming problem, the discovery of novel active compounds against new target is a matter of urgency. Thus *M.oleifera* could become promising natural antimicrobial agent with potential application in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

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