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COMPARATIVE STUDY OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF PLANT EXTRACT - AMLA [*Phyllanthus emblica* L.] TULSI [*Ocimum tenuiflorum* L.] NEEM [*Azadirachta indica* A.JUSS]

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ABSTRACT

Methanolic extracts of dried leaves of *Ocimum tenuiflora*, *Azadirachta indica*, *Phyllanthus emblica* were used for the comparative study of antibacterial and antioxidant activity. The antioxidant activity of these extracts were determined by DPPH [1, 1-Diphenyl-2-picryl hydrazyl] assay. It was found that fraction IV of Tulsi shows more antioxidant activity when compared to Neem and Amla [T>N>A]. These extracts were further tested for antibacterial activity by spread plate method against *Bacillus subtilis, Streptococcus aureus*. It was found that Gram negative bacteria were largely inhibited by the fraction III of Tulsi than that of Neem and Amla against reference antibacterial drug like Tetracyclin. The zone of inhibition was measured which shows that fraction III of Tulsi is having high antibacterial activity when compared to Neem and Amla.

Keywords: Dried leaves of Neem, Tulsi, Amla, Antibacterial activity, Ascorbic acid, DPPH.

INTRODUCTION

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants exists within the body which many of them are derived from dietary sources like fruits, vegetables and teas (Effat Souri*a,* 2007*).* It has been established that oxidative stress is among the major causative factors in the induction of many

chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppressant, neurodegenerative diseases and others.[1-6] The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders. In healthy individuals, the production of free radicals is balanced by the anti oxidative defense system; however, oxidative stress is generated when equilibrium favors free radical generation as a result of a depletion of antioxidant levels. The oxidation of lipid, DNA, protein, carbohydrate, and other biological molecules by toxic Reactive oxygen species may cause DNA mutation or/and serve to damage target cells or tissues, and this often results in cell senescence and death (Lie-Fen Shyura, 2005).

Plant derived drugs remains important resource especially in developing countries, to combat serious disease. Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002; Zhang, 2004). In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). And natural products play on important role in drug development programmes in the pharmaceutical industry (Baker *et al.*, 1995). Currently, there is a growing interest toward natural antioxidants and natural antimicrobials of herbal resources.[10-12] Epidemiological and in vitro studies on medicinal plants strongly supported that plant constituents with antioxidant and antimicrobial activity are capable of exerting protective effects against oxidative stress in biological systems and antibacterial activity against human bacterial species.[13-16] In the present study, three medicinally important plant viz., Amla (*Phyllanthus emblica* L.) belongs to family *Phyllanthaceae*, Tulsi (*Ocimum tenuiflorum* L.) belongs to family *Lamiaceae* and Neem (*Azadirachta indica* A. Juss) belongs to family *Meliaceae*. All the three plants have been used for treatment of several disorders such as common cold, scurvy, cancer and heart diseases. In the present study, the three plants were tested to check the potentiality for antioxidant and antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Plant Materials

Fresh and healthy leaves of Amla (*Phyllanthus emblica* L), Tulsi (*Ocimum tenuiflorum* L.) and Neem (*Azadirachta indica* A. Juss) were collected from different location of Bangalore city. The seed materials were, identified and authenticated from Botanist, Bangalore University, Bangalore. The leaves were washed thoroughly with sterile distilled water and dried in room temperature for two to three days and preserved until further use.

Preparation of Extract

Dried leaves of Amla, Tulsi and Neem were powdered separately and 2.5 grams of each powder was extracted in 42 ml of 80% methanol using waring blender and kept for thirty minutes and centrifuged at 10000 rpm for fifteen minutes. The obtained pellet is extracted with ethyl alcohol (5 Vol). This gives the filtrate of polysaccharide, fats and

waxes (Fraction I). The supernatant obtained is evaporated to 1/10 volume and acidify using 2M sulphuric acid and extracted with chloroform, this gives chloroform extract and aqueous acid layer. The chloroform extract contains phenolics (Fraction II) and aqueous acid layer is basify to pH 10 with ammonium hydroxide and extracted with chloroform – methyl alcohol (3:1) which gives the basic extract containing alkaloids (Fraction III). These different fractions were used to determine *in vitro* antioxidant and antibacterial activity.

Chemicals used

Standard ascorbic acid, methyl alcohol, ethyl alcohol, DPPH [1, 1 - Diphenyl – 2 – picryl hydrazyl]. Chloroform, 2 M sulphuric acid, ammonium hydroxide. All the above chemicals are purchased from chemfort (India) pvt.ltd.

Free radical scavenging activity by DPPH method

Free radicals scavenging potential of all the four fractions of Amla, Tulsi and Neem were tested against a methanolic solution of 1, 1 – Diphenyl – 2 – picryl hydrazyl [DPPH]. Antioxidants reacts with DPPH and convert it to 1, 1 – Diphenyl – 2 – Picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the extract. The change in the absorbance produced at 520 nm has been used as measure of Antioxidant activity. Stock solution of different fractions of Amla, Tulsi and Neem were mixed with DPPH methanol solution [2.5 ml extract + 0.5 ml DPPH] in 3ml of total reaction mixture and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 520 nm and converted to percent antioxidant activity. For a comparative study, the ascorbic acid (40 microgram per ml) was used as the standard. The percentage inhibition activity was calculated by using a formula.

Percent Inhibition = 100 (Absorbance of Blank - Absorbance of Sample) / Absorbance of Blank

Antibacterial Activity

Test organisms

Two pathogenic bacteria namely *B. subtilis* and *S. aureus* were collected from Department of Microbiology, Don Bosco Institute of Biosciences, Kumbalagodu, Bangalore-74. The obtained cultures were brought to the laboratory and subcultured on nutrient agar medium. After 24 hours of incubation at 37°C the cultures were preserved aseptically in refrigerator until further use.

Preparation of Inoculum

A loopful of *B. subtilis* and *S. aureus* was taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test-tube were incubated at 37°C for 24 hours. The broth was standardized using sterile normal saline to obtain a population of 10 cfu/ml.

Preparation of Solvent Extract

One gram of completely evaporated different four fractions of all the three plants was dissolved in 9 ml of methanol. The sterile nutrient agar medium in petridishes was uniformly smeared with test culture. To each well in petriplate, 50 µl of solvent extracts dissolved in methanol were added. The centre well methanol is added which serves as control. For each treatment six replicates were maintained.

Agar Cup Diffusion Method

Agar cup diffusion method described by Shaikh[14] was employed. An overnight culture of *B. subtilis* and *S. aureus* was standardized to contain approx. 10 cfu/ml was inoculated into 20 ml of molten nutrient agar. The culture medium was allowed to set.

Thereafter, a sterile cork borer of 5.0 mm diameter was used to punch wells in the seeded nutrient agar. Five wells were made in the petriplate containing media (One in centre and Four at the border), the agar plugs were removed with a flamed and cooled wire loop. For each well 50 µl of different fractions of Amla, Tulsi and Neem extract were added. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured millimeter. The experiments were repeated for six times.

RESULTS AND DISCUSSION

Among the four fractions of extract tested for antioxidant activity, Fraction IV showed a maximum antioxidant activity and recorded 78.9% Tulsi, 80.6% Neem and 81.4% Amla compared to standard ascorbic acid it recorded 82.3% . Fraction IV showed a significant antioxidant activity and recorded 76.8 % Tulsi, 76.9% Neem and 79.2% Amla. And fraction I and fraction IV recorded less activity. With this observation it was concluded that fraction III which is showing a maximum antioxidant activity and percentage is nearer to standard. It was also observed that the Fraction III contains the terpenoids there is an urgent need for phytochemical analysis to identify the active principles responsible for antioxidant activity. Compared to Fraction III, the other three fractions also showed a satisfactory antioxidant activity which needs the phytochemical analysis. There are many reports available in which the active principles particularly bioactive compounds show antioxidant activity. The antibacterial activity of seed extract also showed a highly significant activity against the two test pathogens *S. aureus and B. subtilis.* (Table 2) shows that Gram negative bacteria shows no zone of inhibition where as Gram positive bacteria shows zone of inhibition for the fractions II, III and IV extracted from Tulsi against reference drug Tetracyclin.

Gram negative bacteria shows zone of inhibition for fraction I where as Gram positive bacteria shows zone of inhibition for fraction III extracted from Neem against reference drug Tetracyclin.

Gram negative bacteria shows no zone of inhibition and Gram positive bacteria shows zone of inhibition for fraction II extracted from Amla against reference drug Tetracyclin From this investigation it was observed that leaves of Tulsi, Neem and Amla have highly potent medicinal value which can be used for medicinal purposes. A further investigation is needed to test the leaves for other human life threatening microbes both fungi & bacteria and also for pharmaceutical uses.

CONCLUSION

The comparative study about antioxidant activity conducted between different medicinal plants like Tulsi, Neem &Amla suggests that Fraction I of all three samples shows no antioxidant activity. Fraction II and IV shows similar antioxidant activity in Amla, and Neem compare to Tulsi. Fraction III shows more antioxidant activity in Amla than Neem and Tulsi. The comparative study about antibacterial activity reveals that Gram negative bacteria shows no zone of inhibition where as Gram positive bacteria shows zone of inhibition for the fractions II, III and IV extracted from Tulsi against reference drug Tetracyclin Gram negative bacteria shows zone of inhibition for fraction I where as Gram positive bacteria shows zone of inhibition for fraction III extracted from Neem against reference drug Tetracyclin. Gram negative bacteria shows no zone of inhibition and Gram positive bacteria shows zone of inhibition for fraction II extracted from Amla against reference drug Tetracyclin.

Table 1: Antioxidant activity of Tulsi, Neem and Amla.

	Tulsi	Neem	Amla
Fraction No.	% Antioxidant Activity	% Antioxidant Activity	% Antioxidant Activity
I	56.8	50.4	44.3
II	60.8	59.4	60.8
III	78.9	80.6	81.4
IV	76.8	76.9	79.2
Std. Ascorbic acid	82.3		

Table 2: Antibacterial activity of different fractions of Amla, Tulsi and Neem

Fractions	Plant Extract					
	Tulsi		Neem		Amla	
	B. subtilis	*S. aureus*	*B. subtilis*	*S. aureus*	*B. subtilis*	*S. aureus*
	Zone of Inhibition (mm)					
I	–	–	+	–	–	–
II	–	+	–	–	–	+
III	–	++	–	+	–	–
IV	–	+	–	–	–	–
Std. Tetracycline	+	+++	+	++	++	++

Zone of inhibition 0.1mm-0.4mm = +**:** 0.5mm-0.9mm = + + **:** >1.0mm = + + +

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