

Antioxidant and Antibacterial Activity of Avocado (*Persea gratissima* Gaertner.) Seed Extract

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Abstract: *In vitro* evaluation of antioxidant and antibacterial activity of solvent extracts of seeds of butter fruit (Avocado) were analyzed. The antioxidant activity of the seed extracts was calculated by using colorimetric method and different fractions of extracts was observed to scavenge 1,1-diphenyl,2-picrylhydrazyl(DPPH) followed the order terpenoids > lipids > alkaloids > polysaccharide. Among the four fractions analyzed, maximum antioxidant activity was observed in fraction III and recorded 81.6%, compared to standard antioxidants ascorbic acid (82.3%). Moderate antioxidant was observed in fraction I, II and IV. Fraction III and IV showed a maximum zone of inhibition against *Bacillus subtilis* and *Staphylococcus aureus* at 50 µl concentration.

Key words: Avocado • Antibacterial • Antioxidant • DPPH • *Bacillus subtilis* and *Staphylococcus aureus*

INTRODUCTION

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions [1]. Bacterial diseases in general and Reactive oxygen species (ROS), in particular such as superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide and peroxynitrite radicals play an important role in oxidative stress related to the pathogenesis of various important diseases[2]. 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, immunosuppression, neurodegenerative diseases and others [3-6]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous or endogenous, whether synthetic or natural, can be

effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [7]. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), Gallic acid esters have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants which show low solubility and moderate antioxidant activity [8]. Among bacterial diseases, *B. subtilis* which causes food borne illness and *Staphylococcus aureus* which cause impetigo, cellulitis and scalded skin syndrome etc. becoming a threatening diseases in humans. Now a days botanicals have been used for treatment or prevention of various human diseases throughout history [2]. Currently, there is a growing interest towards natural antioxidants of herbal resources [9, 10]. An *in vitro* studies on medicinal plants strongly

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supported this idea that plant constituents with antioxidant and antibacterial activity are capable of exerting protective effects against oxidative stress in biological systems [7]. In the present study, the seeds of Avocado (*Persea gratissima* Gaertner) commonly called butter fruit belongs to family *Lauraceae* tested for its potentiality as antioxidant agent as well as its significance in inhibiting bacterial strains. Avocado are a commercially valuable fruit and are cultivated in tropical climates throughout the world. Seed extract of Avocado were selected and tested against multi-drug resistant bacteria *B. subtilis* and *S. aureus*.

MATERIALS AND METHODS

Plant Material: Fresh and healthy butter fruit seed were collected from vegetable and fruitmarket in Bangalore on February 2010. The seed material were washed thoroughly with sterile distilled water, identified and authenticated from Botanist, Bangalore University, Bangalore.

Preparation of Extract: 40 grams of butter fruit seeds made into small pieces and subjected to homogenization for five minutes in methyl alcohol-water [4:1] mixture. The obtained solvent was filtered (Fraction I). The collected residue is extracted with ethyl alcohol (Five times more than the weight of residue) and filtered (Fraction II), the resultant residue contains polysaccharide and the filtrate contains fats and waxes. The filtrate obtained in first step is evaporated and acidified with 2M sulphuric acid and extracted three times with chloroform, this results in formation of an organic layer at lower phase and aqueous acid layer at upper phase. Chloroform extract contains Terpenoids (Fraction III). The aqueous acid layer is basify to pH 10 with ammonium hydroxide and extracted with chloroform-methyl alcohol mixture (3:1) which gives the basic extract that includes alkaloids (Fraction IV). The obtained four fractions were used to determine *invitro* antioxidant and antibacterial activity [11].

Free Radical Scavenging Activity by Dpph Method: Free radical scavenging potential of different fractions of seed extract were tested against 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 520nm has been used as measure of antioxidant activity. Stock solution of different fraction were mixed with DPPH methanol solution [0.5ml, 0.1mM]

in 3 ml of total reaction mixture and allowed to react at room temperature. After 30 minutes, absorbance values were measured at 520nm. All the result obtained was compared to standard antioxidant ascorbic acid (5microgram per ml). The percentage inhibition activity was calculated using a formula [12].

$$\% \text{ of Antioxidant Activity} = \frac{1 - \text{Absorbance of test compound}}{\text{Absorbance of control compound}} \times 100$$

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 10ml 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 10⁴cfu/ml [13].

Preparation of Solvent Extract: One gram of completely evaporated different four fractions was dissolved in 9ml of methanol. The sterile nutrient agar medium in petridishes was uniformly smeared with test culture. To each well in petriplate, 50 il of solvent extracts dissolved in methanol were added. To the centre well methanol is added which serves as control. For each treatment six replicates were maintained [13].

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. 10⁴cfu/ml was inoculated into 20ml of molten nutrient agar. The culture medium was allowed to set. Thereafter, a sterile cork borer of 5.0mm 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 seed extract of butter fruit 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 [13].

Table 1: Antioxidant activity of different fractions of seeds of Butter fruit (Avocado)

Antioxidants	Vol. of seed solvent extract	Vol. of 0.1 mM DPPH	O.D at 520 nm	% of Antioxidant activity
Fraction I	2.5 ml	0.5 ml	0.35	60.8
Fraction II	2.5 ml	0.5 ml	0.28	78.9
Fraction III	2.5 ml	0.5 ml	0.20	81.6
Fraction IV	2.5 ml	0.5 ml	0.35	56.8
Ascorbic acid	2.5 ml	0.5 ml	0.33	82.3

Table 2: Antibacterial activity of different fractions of seed of Butter fruit (Avocado)

Butter fruit seed extract (50 µl concentration)	Zone of Inhibition(mm)	
	<i>S. aureus</i>	<i>B. subtilis</i>
Fraction I	-	-
Fraction II	-	-
Fraction III	30.5 ^b ±0.1	32.7 ^b ±0.1
Fraction IV	20.8 ^a ±0.3	16.7 ^a ±0.2
Ampicillin	32.5 ^c ±0.1	34.5 ^d ±0.2
Chloramphenicol	34.0 ^d ±0.1	33.6 ^d ±0.1

RESULT AND DISCUSSION

Among the four fractions of seed extract of Avocado tested for antioxidant activity, Fraction III showed a maximum antioxidant activity and recorded 81.6% compared to standard ascorbic acid (82.3%). Fraction II showed a significant antioxidant activity and recorded 78.9% and fraction I and fraction IV recorded 60.8 and 56.8% respectively. 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 (Table 1). 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 compound shows antioxidant activity [15-18]. The antibacterial activity of seed extract also showed a highly significant activity against the two test pathogens *S. aureus* and *B. subtilis*. Fraction III recorded 30.5mm inhibition for *S. aureus* and 32.7mm for *B. subtilis* at 50 µl concentration, compared to standard antibiotic ampicillin and chloramphenicol (32.5 and 34.0mm) for *S. aureus* and 34.5 and 33.6mm inhibition for *B. subtilis* respectively. Fraction IV also showed a moderate inhibition of 20.8 and 16.7mm for *S. aureus* and *B. subtilis* respectively (Table 2). From this investigation it was observed that seeds of Avocado have highly potent medicinal value which can be used for medicinal purposes. A further investigation is needed to test the seed for other human life threatening fungi and bacteria [19-21] and also for pharmaceutical uses.

CONCLUSION

The seeds of Avocado (*Persea gratissima*) commonly called as butter fruit belongs to family *Lauraceae* are rich sources of antioxidants and has the highly potent antibacterial activity. The fractions can be further subjected for phytochemical analysis in order to obtain highly efficient concentrated pure antioxidant and antibacterial compounds that can be used for further purification or provide protection from oxidative stress and prevent deterioration of food ingredients and to protect from diseases in humans. Avocado are an edible fruit and the seeds which are considered as waste can be utilized for curing many human diseases and plant diseases. As a edible and natural resources, seeds can be readily used for curing many more diseases and also to identify the antioxidant and antibacterial drugs which will be helpful for human shelf life.

ACKNOWLEDGEMENT

The authors wish to thank Don Bosco Institute of Biosciences and Oxford college of Sciences for providing the lab facilities to conduct the work.

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