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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF SELECTED MANGO (*MANGIFERA INDICA* L.) AND AVOCADO (*PERSEA AMERICANA*) FRUITS IN ILLU ABABOR ZONE, OROMIA REGIONAL STATE, ETHIOPIA

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ABSTRACT

Mango (*Mangifera indica* L.) and Avocado (*Persea americana*) fruits are rich in bioactive molecules that protect human cells against the detrimental effect of free radicals. The phytochemical analysis revealed the Presence of alkaloids, terpenoids, saponins, tannins, phenolics and flavonoids in both fruits. The free radical scavenging activity using total antioxidant capacity of the phosphomolybdenum method and hydrogen peroxide method were carried out on the water extracts of mango and avocado fruits. The order of antioxidant potential according to phosphomolybdenum method and hydrogen peroxide method was found to be higher in *Mangifera indica* L. than in *Persea americana*. The result for the total antioxidant capacity of the measured concentration (0.1 - 0.3 mg/ml) had *mangifera indica* showing the higher capacity with 0.372 mg Ascorbic Acid Equivalence (AAE) and *Persea americana* be with 0.265 mg Ascorbic Acid Equivalence (AAE). Further, the hydrogen peroxide scavenging activity was recorded maximum for *Mangifera indica* L. (70.67%) and (68%) for *Persea americana*. Phytochemical screening on the extracts revealed the presence of alkaloids, phenolics, terpenoids, flavonoids, saponins, and tannins. The results revealed that the antioxidant activity of mango fruit extracts is greater than that of avocado. The antioxidant activities of the same fruits type differ from place to place even in the same zone.

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INTRODUCTION

Mango (*Mangifera indica* L.), Anacardiaceae family, is one of the most popular tropical fruits, followed by banana, pineapple, papaya and avocado. Mangoes are grown in tropical and subtropical regions of the world with India leading world production. The fruit is considered the King of all fruits. Besides, the fruit is rich in antioxidants and, therefore, reduces the risk of cardiac disease, anticancer and antiviral activities [1]. Chemical composition of mango fruits differs with regard to different cultivars and area of production. In Ethiopia, mangoes are both popular and valuable fruits. The success of mango cultivation in Ethiopia could be attributed to the diverse environmental conditions across the country, which extends the fruiting season to eleven months a year. This plant has parts, as the stem bark, leaves and fruit pulp, which are known for various biomedical applications, including the free radicals elimination [2], anti-inflammatory [3] and anticancer [4].

Avocado (*Persea americana*) is an important source of bioactive molecules that protect human cells against the detrimental effect of free radicals. The main antioxidants present in avocado pulp are the oxygenated carotenoids. In addition, avocado contains peresone A and B which are bioactive molecules that protect against inflammation and carcinogenesis. According to the American Diet Association (ADA), avocado can be classified as a functional food due to its high nutritional value and proven beneficial effects for human health [5].

In recent years, there has been a growing interest in functional food that can provide not only the basic nutritional and energetic requirements but also additional physiological benefits. A functional food can be defined as the food that produces a beneficial effect in one or more physiological functions, increases the wellbeing or decreases the risk of a particular disease [6]. Among the functional ingredients, the group most widely studied is the family of antioxidants. Antioxidants are substances that when present at low concentrations, compared with those of the oxidizable substrate significantly delay or inhibit oxidation of that substrate. Traditionally, these compounds have played an important role in food science and technology because of their usefulness as a preservation method against oxidative degradation of foods and scavenging the free radicals [7-8].

Many of today's health problems such as cancer, atherosclerosis, cardiovascular diseases, aging and inflammatory diseases are believed to be due to oxidative damage of vital biomolecules induced by free radicals [9]. Therefore, it is useful to know phytochemical compounds they have and see the antioxidant activities of each mango and avocado fruits.

The benefit of the research is to give information about the health beneficial effects of these fruits. In this study; phytochemical and the antioxidant capacity of the mango and avocado fruit grown in Illu Ababor zone was studied as it aims specifically; to qualitative determination of phytochemical compounds, the reducing power of mango and avocado fruits, to compare the antioxidant capacity of mango and avocado fruits, to suggest possible avenue towards health promotion by observing the scavenging ability and the total antioxidant capacity of mango and avocado fruits.

The current work focuses on the phytochemical screening and study of antioxidant activity of few selected mango and avocado fruits varieties obtained from Illu Ababor zone, Oromia regional state, South West Ethiopia. The need to study this research is to undertake qualitative phytochemical screening and evaluate antioxidant activity of few mango and avocado fruits grown in Illu Ababor zone; Oromia regional state. This is because higher antioxidant capacity of fruits lowers the risk of chronic diseases. The antioxidant activities of some fruits were studied in Mexico, Florida, Singapore and other countries by different methods. In the case of our country, to the best of our knowledge, no research has been done on antioxidant capacity of the mango and avocado fruits varieties grown specifically in Illu Ababor zone, Oromia regional state, South West Ethiopia.

MATERIALS AND METHODS

Chemicals and Standards

Chemicals and standards used include: Methanol, ethanol, hydrochloric acid (HCl), Distilled water, ferric chloride, Ascorbic acid (vitamin C), sodium phosphate, ammonium molybdate, Sulphuric acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, *n*-butanol, ferrous chloride, ceric sulphate, ammonia, Draggendroff's reagent, chloroform, aluminium chloride, olive oil and hydrogen peroxide. All chemicals used were of analytical grade.

Equipments used include: Analytical balance, 0.0001 g or 0.00001 g sensitivity, Blender (warring blender with 1 litre container, Ultra Turrax UT-25 homogenizer, Centrifuge, min 4 × 100 ml tubes and 4000 rpm operating speed, Incubation chamber and UV- visible spectroscopy.

Preparation of sample for the analysis

Sampling and Sample Preparation

The whole mango and avocado samples were obtained from three woredas of Illu Aba Bora region. The collected fruits were washed immediately under tap water and then drained on tissue paper before physical measurements. The collected mango and avocado fruits from the same woreda were separately homogenized. The edible portion of the fruits (16 gm) was homogenized with 50% aqueous: ethanol (32 ml). The homogenate was allowed to stand at room temperature for 30 min with occasional agitation. The extract was centrifuged at 2,000x g for 15 minutes and the supernatant collected was used. From each woredas triplicate sample were transported to laboratory for analysis.

Chemical analysis

Phytochemical Screening

The phytochemical screenings testing for the presence of phytoconstituents were performed using the standard procedures.

Test for alkaloids

For the test of alkaloids, the TLC card having spots of the studied samples were sprayed with Dragendroff's reagent. The appearance of orange colour which indicated the presence of alkaloids was observed.

Test for terpenoids

Two methods were used to test presence of terpenoids.

First, Ceric sulphate solution was sprayed on TLC card having spots of samples. TLC card was heated on a TLC heater. The appearance of brown colour indicated the presence of terpenoids. Second, 3 ml of concentrated H₂SO₄ was carefully added to form a layer to 0.5 g of each of the extract 2 ml of chloroform. A reddish brown colouration of the interface indicated the presence of terpenoids.

Test for saponins

A 5 ml of distilled water was added to 0.5 g of extract in a test tube. The solution was vigorously shaken and stable persistent froth was observed. The frothing was mixed with 3 drops of olive oil and shaken vigorously and formation of an emulsion was observed.

Test for tannins

5 ml of *n*-butanol-HCl solution (1:1 in volume) was added to 2 ml of sample in test tube. A mixture was warmed for 1 hour at 95°C in a water bath. A red colour which indicates the presence of tannins appeared.

Test for phenolics

Neutral ferric chloride was added to each fraction of the sample. A bluish green colour which indicates presence of phenolics appeared.

Test for flavonoids

Two methods were used to test for flavonoids. First, 2 N dilute ammonia (5 ml) was added to a 1ml portion of sample solution in water. 1 ml concentrated sulphuric acid was added. A yellow colouration that disappeared on standing indicated the presence of flavonoids. Second, A few drops of 1% aluminum chloride solution were added to 2 ml of each sample solution. A yellow colouration that indicated the presence of flavonoids was appeared.

Antioxidant Assays of the mango and avocado fruits

The following analytical antioxidant assays were performed for the fruit extracts.

Determination of total antioxidant capacity by phosphomolybdenum complex method

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described by [46]. The assay was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid p^H. A 0.6 ml extract (sample) was combined with 6 ml of reagent solution (2 ml sulfuric acid, 2 ml sodium phosphate and 2 ml ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then its absorbance was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Mean values from three independent samples were calculated for both extracts. Ascorbic acid was used as positive reference standard. Methanol (0.6 ml) were used in the place of extract as the blank. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

Scavenging of Hydrogen Peroxide (H₂O₂)

The hydrogen peroxide scavenging assay was carried out following the procedure of [10]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (p^H 7.4). Different concentrations of the extracts were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide was determined at 230 nm after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a positive control. The percentage scavenging of hydrogen peroxide of extract and standard compounds were calculated using the following formula: % scavenged [H₂O₂] = [(A₀-A₁)/A₀] x 100 where A₀ is the absorbance of the control, and A₁ is the absorbance in the presence of the sample.

RESULT AND DISCUSSIONS**Determination of Major Phytochemicals**

The medicinal values of fruits are dictated by their phytochemicals and other chemical constituents. Major groups of phytochemicals were detected by standard colour tests and summarized (Table 1). In qualitative analysis of homogenized extracts of both Mango (*Mangifera indica* L.) and avocado (*Persea americana*) fruits exhibited positive results for six phytochemical tests.

Table 4.1: Test of Phytochemicals in Mango (*Mangifera indica* L.) and avocado (*Persea americana*) fruits.

Phytochemicals	Mango (<i>Mangifera indica</i> L.)	Avocado (<i>Persea americana</i>)
Alkaloids	+	+
Terpenoids	+	+
Saponins	+	+
Tannins	+	+
Phenolics	+	+
Flavonoids	+	+

Remark: (+) = Present.

The phytochemical analysis revealed the Presence of alkaloids, terpenoids, saponins, tannins, phenolics and flavonoids. The results were consistent with several previous studies on the chemical content of Mango (*Mangifera indica* L.) and avocado (*Persea americana*) fruits.

Persea americana showed positive results for all the phytochemical tests agreeing with previous analysis and *Mangifera Indica* showed the presence of all other phytoconstituents tested agreeing with previous analysis except the presence of alkaloids in this study.

From the screening tests done it can be deduced that the plant samples may show high total antioxidant activity due to the presence of flavonoids and tannins. Phenolic compound constituents like tannins and flavonoids act highly as antioxidants (protection and regeneration of other dietary antioxidants) and free radical scavengers. Flavonoids and tannins are likely to be responsible for the free radical scavenging activities because they are phenolic compounds which in turns are good primary antioxidants or free radical scavengers.

Antioxidant Assay

Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the description of ROS attack, by scavenging reactive metabolites & converting them to less reactive molecules.

Recently, there are increasing evidences that indigenous antioxidants are becoming useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in medicinal plants, fruits and vegetables.

The *in vitro* antioxidant activity of different mango and avocado fruits were determined and the results were analyzed.

Determination of total antioxidant capacity by phosphomolybdenum complex method

The measure of the ability of substances extracted from fruits to delay oxidative stress in a controlled system which is defined as Total Antioxidant capacity (TAC) is described in (Table 3.2).

Table 3.2: Determination of total antioxidant capacity of mango and avocado fruits by phosphomolybdenum complex method.

S.No	Fruits		Total antioxidant capacity (AAE mg/25g)
1	Mango	Nopha	$0.372 \pm 3.45 \times 10^{-3}$
		Mettu	$0.360 \pm 3.55 \times 10^{-3}$
		Gore	$0.356 \pm 3.76 \times 10^{-3}$
2	Avocado	Nopha	$0.292 \pm 7.33 \times 10^{-5}$
		Mettu	$0.274 \pm 6.93 \times 10^{-5}$
		Gore	$0.265 \pm 7.25 \times 10^{-5}$

The extracts of *Mangifera indica* L. showed different range of extent of antioxidant activity from that of *Persea americana* and this can be related to the high amounts of flavonoids and phenolic compounds in extracts [11]. All extracts gave a positive test for flavonoids with different intensity to the test hence the indication of varying antioxidant capacity since the amount of phenolic compounds in the extracts could be different from one extract to the other.

From table 4.2, the results showed highest antioxidant capacity of 0.372 mg AAE/DW with SD of 3.45×10^{-3} for *Mangifera indica* and 0.265 mg AAE/DW with SD as 7.25×10^{-5} for *Persea americana* showing the lowest TAC.

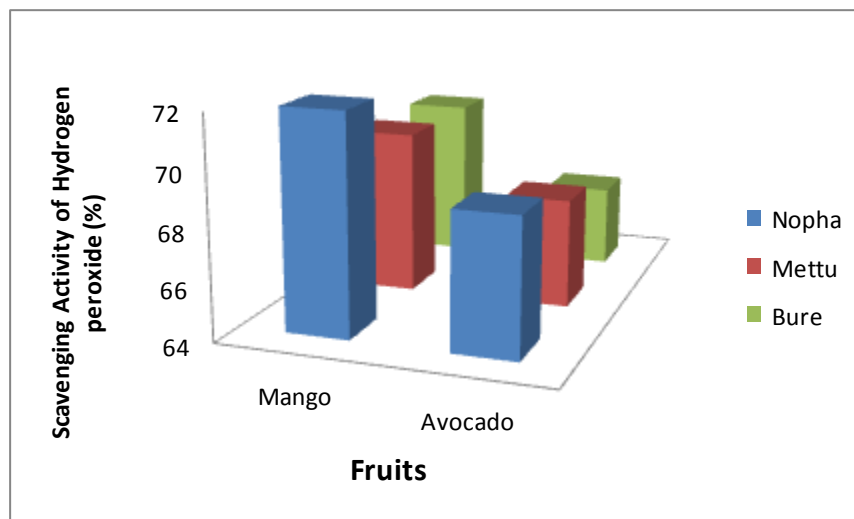
This shows the same trend with *Mangifera indica* having a higher total antioxidant capacity than *Persea americana* with 0.372 and 0.265 Ascorbic Acid Equivalence (AAE) respectively With respect to antioxidant capacity, the results of [12] and [11] showed high antioxidant capacity for *Mangifera indica* and *Persea americana*.

Scavenging of Hydrogen peroxide (H₂O₂)

The ability of various mango and avocado fruits to scavenge Hydrogen peroxide was determined [Table 4.3 and Figure 4.1]. Percentage scavenging ability was higher for *mangifera indica* L. with 70.67% and lowest for *persea americana* 68 % both at the concentration of 3 mg/ml. This is the highest percentage of free radicals that can be reduced at the experimental concentrations.

Table 4.3: Scavenging Activity of Hydrogen peroxide (H₂O₂) of various mango and avocado fruits.

S.No	Fruits		Scavenging Activity of Hydrogen peroxide (%)
1	Mango	Nopha	72
		Mettu	70
		Gore	70
		Nopha	69
2	Avocado	Mettu	68
		Gore	67

**Figure 4.1: Scavenging Activity of Hydrogen peroxide (H₂O₂) of various mango and avocado fruits.**

In vitro antioxidant activity was determined by two different procedures and it has been determined that aqueous extracts of these mango (*Mangifera indica*) and avocado (*Persea americana*) fruits possessed maximum antioxidant activity in reference to standard antioxidant.

The results presented indicate that *Mangifera indica* fruit extracts show better antioxidant activity than *Persea americana* fruit extracts and samples from Nopha (both mango and avocado fruit) shows best antioxidant activity and Mettu samples were proved to be the second while Bure samples were with least value of antioxidant activity both by phosphomolybdenum complex method and hydrogen peroxide method. However, all results revealed that these fruits are of great importance due to their high antioxidant activity. The difference shown by these samples from different woredas of Ilu Aba Bora zone may be due to some factors like topographic difference and agricultural practices which can lead to the difference of level of phytochemical compounds. Thus it can be said that these fruits are useful for consumption and are beneficial for health.

CONCLUSION

Both fruits screened for phytochemical constituents seemed to have potential of being good antioxidant. *Mangifera indica* fruit extracts show better antioxidant activity than *Persea americana* fruit extracts and the value of antioxidant activity of different samples of the same fruit shows difference. In general; *Mangifera indica* has shown greater antioxidant activity than *Persea americana* regardless of the sample area. Both fruits gave positive test for all phytochemical compounds tested.

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