Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):116-121

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical analysis and antioxidant activity of *Arachis hypogea*

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ABSTRACT

The objective of the present study was to evaluate the phytochemical constitution and antioxidant activity of ethanolic extract of Runner and Spanish variety groundnuts in three different forms such as Raw (dry), Boiled and Roasted. Qualitative analysis of phytochemical constituents namely tannins, phlobatannins, saponins, flavonoids, quinones, coumerin, terpenoids and cardiac glycosides were performed by standard protocols. The antioxidant activity was analyzed by DPPH and ABTS radical scavenging assays. The phytochemical screening showed the presence of tannins, phlobatannins, saponins, flavonoids, quinones, terpenoids and cardiac glycosides in both the Runner and Spanish variety groundnuts. The numerous bioactive components in peanut contribute to their antioxidant capacity. The antioxidant level of roasted were found to be higher than boiled and raw in both Spanish and runner variety in DPPH and ABTS, Whereas the level of antioxidant of boiled were found to be higher than raw in DPPH assay ,but the level of boiled groundnuts were found to be lower than raw in ABTS assay.

Key words: Runner, Spanish, antioxidant, Saponin, Flavonoids, phytochemicals, Bioavailability, reactive oxygen species, Oxidative stress

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INTRODUCTION

Legumes are rich in protein, calories, certain minerals and vitamins. Among the legumes, nuts are a good source of oil containing higher amounts of unsaturated fatty acids as compared to saturated fatty acids. Legumes also contain some of the phytochemicals which have dual effect on human health. Peanuts (Arachis hypogaea) or groundnut are the world major oilseed crops. The only nut that grows on ground is the groundnut, often called as “The King of Oilseeds”, and is the important inexpensive source of protein, fat, minerals, and vitamins in the diets of rural populations, especially children[1]. Groundnut is commonly called as poor man’s nut [2, 3]. Major proportion of the peanut produce is processed for direct consumption as peanut butter, salted peanuts, and confectionery; whereas in Asia, particularly in India, peanuts are utilized mainly for oil production [4].India is one of the largest producers of groundnut. The export of groundnut from India is continuously increasing. Groundnuts are classified into four market-types (Runners, Spanish, Virginia and Valencia). The chemo preventive action of plant foods has been attributed to the presence of some biologically active phytochemicals. Groundnut seed contains 44 to 56% oil and 22 to 30% protein on a dry seed basis and is a rich source of minerals (P, Ca, Mg and K) and Vitamins (E, K and B group) [5]. Groundnut provides considerable amounts of mineral elements to supplement the dietary requirements of humans and farm animals. [6]. Groundnuts are one of those plant foods that are a dietary source of phytochemicals. [7]. Array of nutrients and phytochemicals play an important role in mechanism responsible for its putative health benefits.Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process. This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and

that dietary antioxidants oppose this and lowers risk of disease. Antioxidant when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance [8]. It is reported that the antioxidant constituents of plant materials provide protection from coronary heart disease and cancer and protect the body from damage caused by free radical induced oxidative stress [9]. The present study is focussed on screening for photochemical using standard techniques for the detection of the saponins, tannins, terpenoids, quinone, coumerin, flavonoids, cardiac glycoside and phlobatannins and antioxidant activity of *Arachis hypogea* in three different forms such as Dry, Boiled, and Roasted.

EXPERIMENTAL SECTION

The two major variety of groundnut that are commonly found in Tamilnadu are Runner and Spanish and hence they were chosen for the study. The groundnuts were bought from the local market in Chennai and the seed were removed. These two varieties have contrast characteristics in terms of seed colour of the seed coat and size of the seed. The Runner variety groundnut has pale pink coloured big sized seeds, whereas the Spanish variety has dark red coloured small sized seeds. The study also includes three forms of the two varieties of groundnut viz., Dry, Boiled (100ºC for 10 min) and Roasted. (85 ºC for 20 min) . The seeds were then broken into coarse granules using mortar and pestle. 50gms were weighed and used for extraction process. The ethanolic extraction was carried out using soxhlet extractor. The groundnut samples were packed in the thimble and was extracted using ethanol, the extraction process was carried out at 74 ^{o} C for 4 hours.

2.1 Phytochemical Screening

The ethanolic extracts of the Ground nuts were subjected to different chemical tests for the detection of different phyto constituents using standard procedures [10, 11, 12].

2.1.1 Test for Tannins:

1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black colouration.

2.1.2 Test for Phlobatannins:

When crude extract of each Ground nut sample was boiled with 2% aqueous HCl the deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

2.1.3 Test for Saponins:

Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

2.1.4 Test for Flavonoids:

5ml of dilute ammonia solution was added to a portion of the crude extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

2.1.5 Test for Quinones:

Dilute NaOH was added to 1ml of crude extract. Blue green or red coloration indicates the presence of quinines.

2.1.6 Test for Coumerin:

10% NaOH was added to the extract and chloroform was added for observation of yellow color, which shows the presence of coumerin.

2.1.7 Test for Terpenoids (Salkowski test):

5ml of extract was mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter–face was formed to show positive results for the presence of terpenooids.

2.1.8 Test for Cardiac glycosides (Keller-Killani test):

5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardinolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.2 In *vitro* Antioxidant activity

2.2.1 1, 1-dephenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the ground nut extracts required to scavenge DPPH showed a dose dependant response. The DPPH assay was carried out after making some modifications in the standard protocol. 1.5ml of o.1mM DPPH solution was mixed with 1.5ml of various concentrations ground nut extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independant assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

Inhibition (%)=$[(A_{control}-A_{test)}/A_{control}]*100$

Where $A_{control}$ is the absorbance of the control (L-Ascorbic acid) and A_{test} is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

2.2.2 ABTS Radical scavenging activity

Monocation radical ABTS+ (2,2'-azinobis(3-ethylbenzothiazoline-6- sulfonic acid) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hr before use. Different concentrations (10-100 μg/ml) of extract or standard (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with methanol to make 1 ml. The absorbance was read at 745nm and the % inhibition was calculated. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical screening was carried out using ethanolic extract on runner and Spanish variety of dried, boiled and roasted seed. The result revealed the presence of phytochemical such as tannin, saponin, phylobatanin, flavonoid, terpenoid and cardiac glycoside in runner variety for all the three form, quinone were found to be absent in all the three forms and coumerin were absent in boiled groundnut as in table I. Whereas in case of Spanish variety tannin, saponin, phylobatanin, flavonoid, terpenoid and cardiac glycoside were found to be present in all the three forms, but quinone was not present in any of the three forms and coumerin was found to be present only in fried groundnut as indicated in table II. Knowledge about phytoconstituents is desirable during the synthesis of complex chemical substances [13, 14, 15]. The increase in biological activity may be due the presence of wide range of phytochemicals.

Qualitative Analysis

Table I: Runner variety Groundnut

Groundnut Type	Dry	Boiled	Roasted
TANNIN	+	+	+
PHLOBATANNIN	+	+	+
SAPONIN	+	+	+
FLAVONOID	+	+	+
QUINONE	-	-	-
COUMERIN	+	-	+
TERPENOID	+	+	+
CARDIAC GLYCOSIDE	+	+	+

Table II: Spanish variety Groundnut

Groundnut Type	Dry	Boiled	Roasted
Solvent	Ethanol	Ethanol	Ethanol
TANNIN	+	+	+
PHLOBATANNIN	+	+	+
SAPONIN	+	+	+
FLAVONOID	+	+	+
QUINONE	-	-	-
COUMERIN	-	-	+
TERPENOID	+	+	+
CARDIAC GLYCOSIDE	+	+	+

Plants produce a very diverse group of secondary metabolites with antioxidant potential. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the aging process [16, 17, 18]. The phytochemicals analysis also confirmed the presence of important bioactive compounds such as Flavonoids and Terepenoids which are considered to be an effective plant derived antioxidants. But there was a significant variation of the antioxidant activity of the processed groundnuts. The antioxidant activity of the groundnuts was analyzed with the help of two assays viz., DPPH and ABTS free radical scavenging activity assays. DPPH is a stable nitrogen centered free radical, which is used to evaluate antioxidant activity because of its short span of radical quenching capacities. In general, DPPH scavenging activities increased with increasing phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes. These phenolic components possess many hydroxyl groups including o-dihydroxy groupwhich have very strong radical scavenging effect and antioxidant power [19]. In the present study, DPPH scavenging activity of runner and spanish variety was found to be high for fried groundnut when compared to boiled and raw as in Fig 1,2,3,4, the antioxidant activity was to increase with increase concentration. The increase in antioxidant activity in roasted form may be due to Maillard reaction product as of Talcott *et al*., 2005[20]. Our result was in accordance with Yu et al. [21] that reported 39.5% increase in peanut skin phenolics after roasting in their study on the effect of processing on peanut skin using 80% ethanol as extraction solvent. Boiling also had a significant effect on the antioxidant activity, the antioxidant activities were also found to high when compared to raw in both the variety. In addition, it has been indicated that effectiveness of processing step to liberate antioxidant compounds from plants may vary depending on species [22]. In ABTS radical scavenging activity, the roasted form of both variety showed higher antioxidant than the standard ascorbic acid that indicate greater reducing power than the standard. Whereas the antioxidant level of raw were higher than the boiled groundnut for both Spanish and Runner variety in ABTS assay (Fig 3&4).

Free radical scavenging activity

Fig1: DPPH Activity of Runner Variety

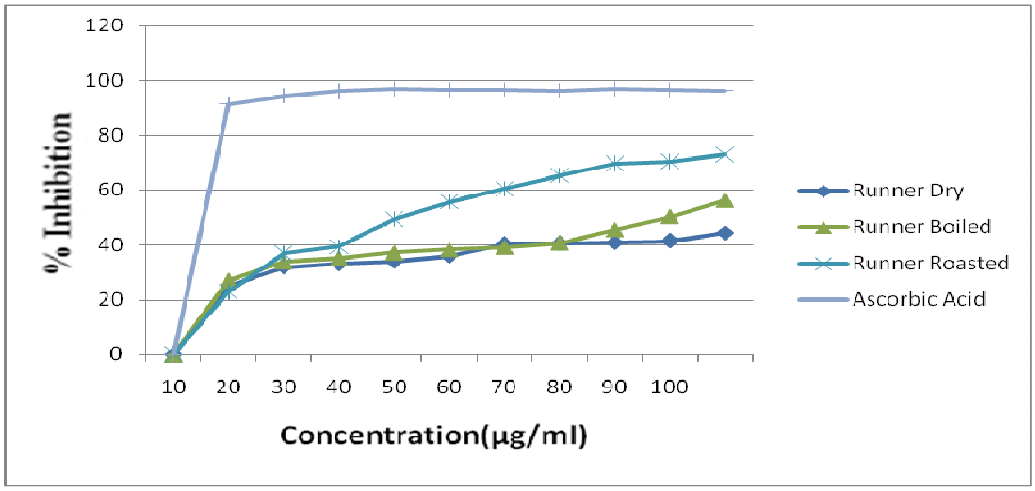


Fig 2: DPPH for Spanish variety groundnut

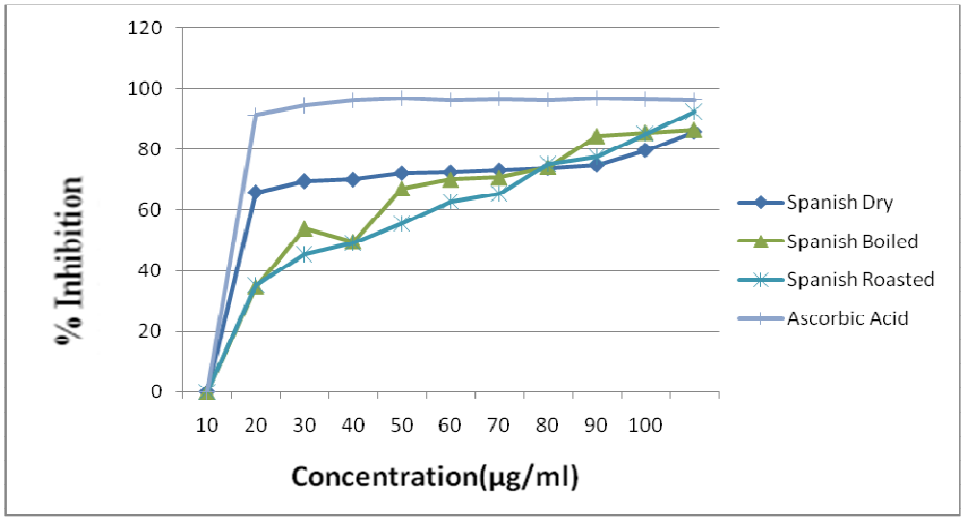


Fig 3: ABTS Scavenging Activity of Runner Variety

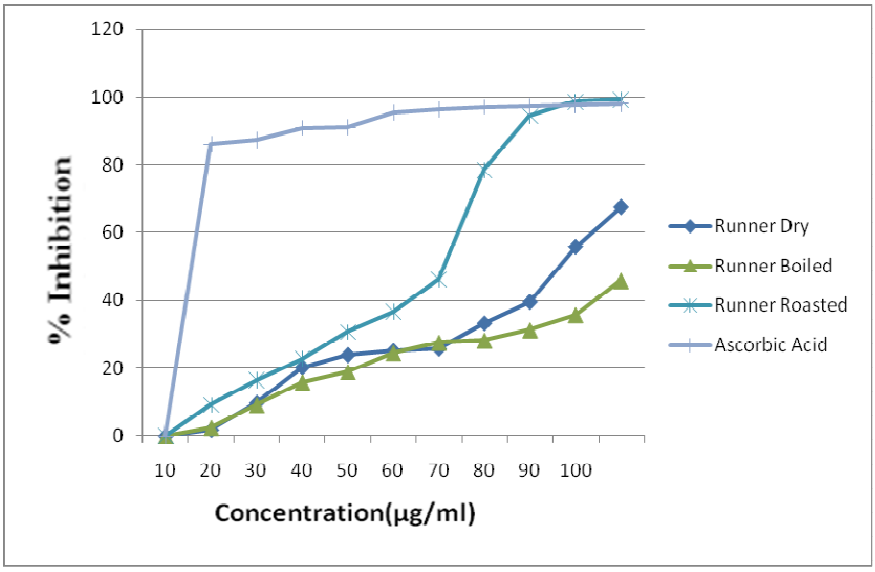
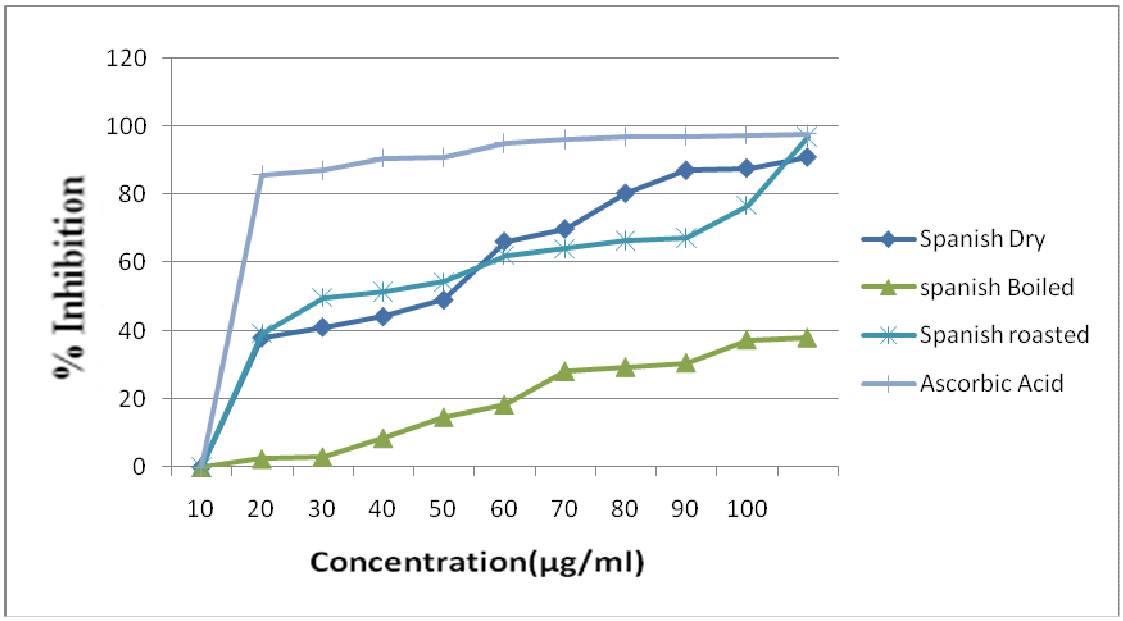


Fig 4: ABTS Activity of Spanish Variety



CONCLUSION

Phytochemical screening of ethanolic extracts *Arachis hypogea* had revealed the presence flavonoids, tannins, terpenoids, saponins, steroids, alkaloids by positive reaction with the respective test reagent. Results obtained in this investigation indicate that Spanish roasted extract, exhibited highest antioxidant and reducing activities. The finding of this study suggests that the groundnut could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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