

**STUDIES ON PHYTOCHEMICAL SCREENING AND *IN VITRO*
ANTIOXIDANT ACTIVITY OF ETHIOPIAN INDIGENOUS
MEDICINAL PLANTS, *ARTEMISIA ABYSSINICA* SCH.BIP. EX A.RICH**

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ABSTRACT

Artemisia abyssinica Sch. Bip. ex A. Rich (Family- Asteraceae; *Chikugn*-Amharic) has a popular traditional medicine for the treatment of various diseases especially among the South-Western people of Ethiopia. The present study was undertaken to find the phytochemical screening and antioxidant potential of *A.abyssinica* leaves and stem by aqueous, methanol and hexane solvents. Preliminary phytochemical investigation was carried out on three extracts by prescribed standard methods. Phytochemical analyses revealed the presence of alkaloids, saponins, flavonoids and tannins. The fraction inhibition of lipid peroxide at the first stage of oxidation illustrated antioxidant activity of *A.abyssinica* leaves and stem as 90% and 88% compared to those of gallic acid (97%) and BHT (84%) respectively. The results of the

present study revealed that *A.abyssinica* leaves and stem extracts have a significant antioxidant capacity and could be expressed as source of antioxidant components.

KEYWORDS: *A.abyssinica*, phytochemicals, Antioxidant activity, traditional medicine.

INTRODUCTION

Medicinal herbs and their specific bioactive compounds are the primary sources of natural products, which produce definite physiological actions on the human body. They are

screened and used to treat for various disease like cancer, diabetes, ageing, neurological diseases and cardiovascular diseases.^[1] Plants containing secondary metabolites like alkaloids, saponins, flavones that can prevent or slow that metabolically produce free radicals. Exogenous sources of free radicals include pesticides, atmospheric pollution, smoking, radiation and organic solvents.^[2,3] The potentially reactive derivatives of oxygen, endorsed as Reactive Oxygen Species (ROS), are spontaneously generated by human cell. These ROS are scavenged by the antioxidants present in the body. Nevertheless, overproduction of ROS and/or insufficient antioxidant can simply cause oxidative damage to biomolecules such as DNA, proteins, lipids and carbohydrates.^[4] Plants containing phenolic compounds are agents of antioxidant, that acts as free radical scavengers^[5], based on the presence of its redox properties, reducing agents and metal chelators.^[6] Many studies show that flavonoids has antioxidant activity and helps to heal various diseases. Hence plant phytochemicals may be most efficient in combating or preventing disease due to their antioxidant scavenging potential.^[7]

Artemisia abyssinica Sch. Bip. ex A. Rich (Family- Asteraceae; *Chikugn*-Amharic) is a short lived perennial, aromatic, grey, silky hairy plant. It is widely distributed in Asia, Saudi Arabia, Yemen, Northeast Tropical Africa, Ethiopia and Eritrea. It is well known stimulant and an analgesic. Leaves are ternate, grey green develop upto 10cm long. It is widely grown in higher altitude from 2000m to 3300m. Stems are sparingly branched that are superiorly grooved. It is used by Ethiopian population for treating headache and mosquito repellent. Traditionally the plant has been used as an antileishmanial, antitrypanosomal, anthelmintic, antispasmodic, antirheumatic and antibacterial agent.^[8]

Based upon ethanobotanical survey of Ethiopian indigenous medicinal plants, *A.abyssinica* has been chosen to prove scientifically having phytoactive compounds and antioxidant activity on *in vitro* studies. The phytochemicals generated data from the three different extracts of these plants may be used as tools for quality control of drugs in the future, for the healing of a diversity of disease conditions.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid, Ferric chloride, HCl, Dragendorff 's reagent, hexane, methanol, gallic acid, chloroform, H₂SO₄, Folin-Ciocalteu reagent, aluminium chloride, potassium acetate, phosphate buffer, K₃Fe(CN)₆, 2-thiobarbituric acid, ferric thiocyanate, butylated hydroxyl toluene, 2,2-diphenyl-1-picrylhydrazyl, potassium persulphate, hydrogen peroxide, sulfanilic acid, glacial acetic acid, potassium metabisulphite, NADH were all purchased from Chemico Glass & Scientific Company, Erode, Tamilnadu, India. All the chemicals used in this experiment were of analytical grade.

Collection and authentication of plant material

The plant of *A.abysinica* was collected from Jimma University Garden, Jimma, South West Ethiopia in the month of September-2014. The plant has been taxonomically identified and authenticated by the Jimma University Botanist Dr. Ramesh Mochikkal and kept in Jimma University Botanical Science and Herbarium for future references.

Preparation of the extract

A.abysinica was collected and air dried under shade and then roughly powdered with the help of mechanical blender. The powder was conceded through filter and stored in an airtight container for the solvent extraction.

Methanol extract of leaves and stem of *A.abysinica*

The shade dried coarsely powdered of leaves and stem of *A.abysinica* was immersed and extracted with methanol for 72hrs. After completion of extraction, the defatted extracts were sieved by Whatmann filter paper No.1 to eliminate any contamination. The extract was intensed by vaccum distillation to decrease the volume; the intensed extract was transferred to 100 ml beaker and the remaining solvent was volatalised. Dark greenish yellow coloured extract was acquired. The intensed extract was then kept in a dessicator to eliminate the unnecessary moisture. The dried extract was packed in air tight glass container for further studies.

Hexane extract of leaves and stems of *A.abysinica*

The marc left after methanol extraction was dried and then immersed and extracted with hexane, upto 72hrs. After completion of extraction, the solvent was removed by distillation. Dark greenish yellow colour residue was obtained. The residue was then stored in a dessicator.

Aqueous extract of leaves and stems of *A.abysinnica*

The marc left after hexane extraction was again dried and then macerated with distilled water in a 2 litre round bottom flask, for 72 hrs. 10 ml of chloroform was added daily to avoid fungal growth. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Green coloured extract was obtained and it was stored in a dessicator to remove the the excessive moisture.

Preliminary phytochemical studies^[9,10]

The extracts obtained (methanol, hexane and aqueous) was subjected to the following preliminary phytochemical studies.

Test for Alkaloids**a) Dragendorff's test**

To 1ml of the extracts, 2ml of distilled water was added, 2 M hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendorff's reagent was added. Formation of orange or red precipitates indicates the presence of alkaloids.

b) Hagger's Test

To 1ml of the extracts were taken in test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirm the presence of alkaloids.

c) Wagners Test

1ml of extracts were acidified with 1.5% v/v of hydrochloric acid and a few drops of wagners reagent were added. A yellow or brown precipitate indicates the presence of alkaloids.

d) Mayers Test

To a few drops of the mayers reagent, 1ml of extracts were added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Test for Carbohydrates**a) Anthrone Test**

1ml of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2ml of anthrone reagent solution was added. Formation of green or blue color indicates the presence of reducing sugars.

b) Benedicts Test

1ml of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 5ml of Benedict's solution was added and boiled for 5mins. Formation of brick red colored precipitate indicates the presence of reducing sugars.

c) Fehlings Test

1ml of extract were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 1ml mixture of equal parts of Fehlings solution A and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicates the presence of reducing sugar.

d) Molischs Test

1ml of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To these 2 drops of freshly prepared 20% alcoholic solution of α -naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red-Violet ring appear, indicating the presence of carbohydrates which disappear on the addition of excess of alkali.

Test for flavonoids

a) Shinods test

1ml of extracts were dissolved in 5ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown color indicates the presence of flavonoids.

b) With con. Sulphuric acid test

Yellow colour (anthocyanins), yellow to orange colour (flavones) and orange to crimson (flavonones).

Test for Glycosides

Molisch Test

1ml of extracts were shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 2-3 drops of Molisch reagent was added, mixed and 2ml of conc. sulfuric acid was added carefully through the side of the test tube. Reddish violet ring appears, indicating the presence of glycosides.

Test for proteins and free amino acids

1. Millions reagent

Appearance of red color shows the presence of protein and free amino acid.

2. Ninhydrin reagent

Appearance of purple color shows the presence of protein and free amino acids.

3. Biuret test

Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution was added.

Appearance of pink or purple shows the presence of proteins and free amino acids.

Test for gums and mucilage

Precipitation with 95% alcohol: Small quantities of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test for anthraquinones

About five ml of the extract solution was hydrolysed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Saponins**Foam test**

In a test tube containing about 5ml of extracts, a drop of sodium bicarbonates solution was added. The test tube was shaken vigorously and left for 3mins. Formation of honeycomb like froth indicates the presence of saponins.

Test for Sterols**a) Liebermann-Buchards test**

1ml of extracts were dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green color indicates the presence of steroids.

b) Salkowski reaction

1ml of extract were shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red color indicated the presence of steroids.

Test for fixed oils

Spot test

Small quantities of various extracts were separately pressed between the two filter papers. Appearance of oil stains on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for triterpenoids

About two ml of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for phenolic compounds and tannins

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

Ferric chloride solutions (5%)-Violet color.

1% solution of gelatin containing 10% sodium chloride-white precipitate.

10% lead acetate solution-white precipitate.

In vitro antioxidant assay

The antioxidant activity of the aqueous plant extract was determined using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxide at the beginning of peroxidation while TBA method was used to measure free radicals present after peroxide oxidation.

Ferric thiocyanate (FTC) method.

The standard method described by Kikuzaki *et al.*^[12] was used for FTC determination. A mixture of 2 ml of sample in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water contained in screw cap vial (Ø38 × 75 mm) was placed in an oven at 40°C in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred to a test tube (Ø38 × 150 mm) and to it; 9.7 ml of 75% (v/v) aqueous ethanol, followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid were added. Three minutes after the addition of ferrous chloride to the reaction mixture,

the absorbance of the resulting mixture (red colour) was measured at 500 nm every 24 h until the absorbance of the control reached its maximum. Butylated hydroxyl toluene (BHT) (final concentration of 0.02% w/v) was used as positive control, while the mixture without the plant extract was used as the negative control.

Thiobarbituric acid (TBA) method

The method of Ottolenghi^[13] modified by Kikuzaki and Nakatani^[14] was used for the determination of free radicals present in the aqueous leaf extract. The final sample concentration of 0.02% w/v from the same samples prepared for FTC assay was used. Two ml of 20% trichloroacetic acid and 2 ml of 0.67% of thiobarbituric acid were added to 1 ml of sample solution from the FTC method. The mixture was placed in a boiling water bath for 10 min and then centrifuged after cooling at 3000 rpm for 20 min. The absorbance activity of the supernatant was measured at 552 nm and recorded after it has reached its maximum.

RESULTS

In the study, preliminary phytochemical investigation has been done in the three extracts (aqueous, methanol and hexane) of *A.abyssinica* leaves and stems showed the presence of phytochemical constituents namely alkaloids, saponins, flavonoids and tannins and absence of anthoquinones, steroids, triterpenoids, aminoacids and glycosides described in Table 1.

Figure 1 illustrated *in vitro* antioxidant assay of the *A.abyssinica* leaves and stem extracts which has significant antioxidant potential compared with standard Gallic acid and BHT. The proportion inhibition of lipid peroxide at the first phase of oxidation showed antioxidant activity of leaves extract of *A.abyssinica* as 90% and 88% compared to those of gallic acid (97%) and BHT (84%) respectively. The percentage inhibition of malondialdehyde by stem extract of *A.abyssinica* showed percentage inhibition of 76% and 70% compared to both BHT (79.24%) and gallic (95.2%).

Table 1: Phytochemical investigation of leaves and stem of *A.abyssinica* using aqueous, methanol and hexane solvents.

Analysis	Leaves			Stem		
	Aqueous	Methanol	Hexane	Aqueous	Methanol	Hexane
Alkaloids	+	-	+++	+++	-	+++
Protein and aminoacids	-	-	-	-	-	-
<i>Anthraquinones</i>	-	-	-	-	-	-
<i>Flavonoids</i>	+	++	++	-	-	-
<i>Glycosides</i>	-	-	-	-	-	-

<i>Saponins</i>	+	+	-	+	+	-
<i>Steroids</i>	-	-	-	-	-	-
<i>Total phenols and Tannins</i>	-	++	++	-	-	-
<i>Triterpenoids</i>	-	-	-	-	-	-

+++ = appreciable amount (positive within 5 mins.); ++ = moderate amount (positive after 5 mins. but within 10 mins); + = trace amount (positive after 10 mins. but within 15 mins); - = completely absent.

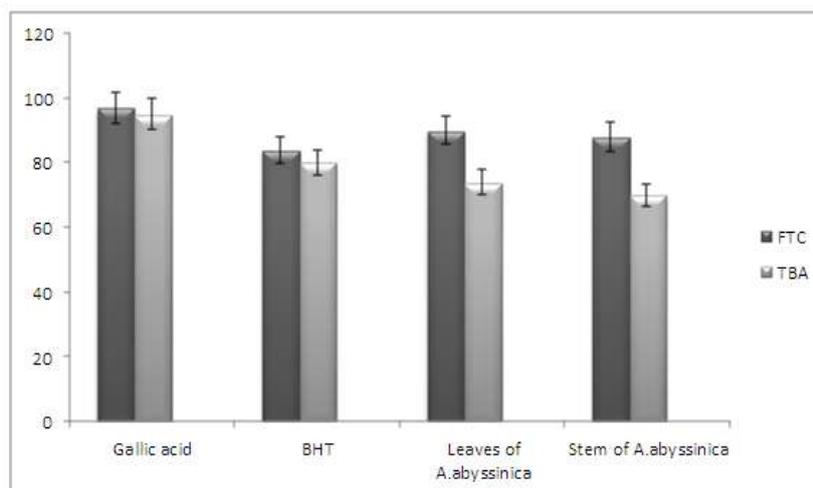


Figure 1 Antioxidant properties of aqueous extract of leaves and stem of *A.abyssinica* compared to the standards (Gallic acid and BHT) as determined with the FTC (500 nm) and TBA (552 nm) methods on the 7th day. TBA: Thiobarbituric acid. FTC: Ferric thiocyanate. BHT: Butylated hydroxyl toluene.

DISCUSSION

Nowadays medicinal plants with antioxidative property have been the major objectives of the investigation. It is alleged that these herbs can nullify or protect from free radical causing tissue damage.^[11] Free radicals and ROS are the primary causative for various human diseases.^[12] The main advantage of these medicinal plants have secondary metabolites, which has main role of additive or synergistic action at single or multiple target sites connected with a pathophysiological process.^[13]

The considerable amount of flavonoids and tannins may have antioxidant potentials, which act as free radical terminators.^[5] Flavonoids are major active nutraceutical ingredients which are nontoxic and scientifically proved components. They are primarily used as either treatment or prevention of diseases.^[14] Plant containing phenolic compounds, that acts as potent antioxidant scavengers and metal chelators.

Phenolic compounds have long been recognized as the potential of antiinflammatory, antihyperglycemic, antiallergic, anticarcinogenic, antimutagenic, hepatoprotective, antithrombotic, antibacterial and antiviral activities by chelating process.^[15] Phenolic compounds naturally contain hydroxyl functional groups, that are primarily accountable for their antioxidant effects.^[16]

CONCLUSION

This study confirms the preliminary phytochemical compounds and *in vitro* antioxidant potential of leaves and stem extract of the *A.abysinica*, with results similar to individual standard such as gallic acid and BHT. Further studies are needed to elucidate the *in vivo* potential of these plants in the treatment of human diseases resulting from oxidative stress.

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