International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-4, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Evaluation of Phytochemical Screening and *In Vitro* Antioxidant Activity of *Cordia Africana* Lam. (Family: Boraginaceae), A Native African Herb

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Manuscript No: IJPRS/V4/I4/00231, Received On: 30/12/2015, Accepted On: 07/01/2016

ABSTRACT

Cordia Africana Lam. is a well known traditional ethnomedicinal use in the treatment of many diseases among African especially the South-east people of Ethiopia. The present study was undertaken to investigate the *in vitro* antioxidant value of aqueous, benzene, CCl_4 and hexane extracts of leaves, stem and fruits of *C.africana* along with finding its phytochemicals screening. *In vitro* antioxidant actions of plant extract was analyzed by measuring its total flavonoid, total phenol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing property. The preliminary phytochemical analysis was carried out on extract based on the standard prescribed method. The results of phytochemical analyses showed the presence of alkaloids, flavonoids, total phenols and tannins. The total phenol and flavonoid contents are 224.78mg gallic acid equivalent/g extract and 335.45mgQuercetin equivalent/g extract respectively. The extracts exhibited significantly high and dose-dependent DPPH radical scavenging and ferric reducing property similar with the respective standards, Quercetin and Ascorbic acid. Established along the present findings, *C.africana* of whole plant possess a noteworthy antioxidant potential and could be shown evidence of as a source of antioxidant additives.

KEYWORDS

Cordia Africana, Preliminary Phytochemical Analysis, *In Vitro* Antioxidant Activity

INTRODUCTION

The detection of medicinal plants are very helpful to human as well as veterinary since natural strands commenced time immemorial. Based on clinical and experimental studies, humans have learnt a variety of uses of the medicinal plants.[1,2] In up to date, emphasize on medicinal herb research has enhanced throughout the world and a note worthy substantiation has collected to confirm massive potential of herbs utilized in an assortment of conventional

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Around 15,000 medicinal herbs have been sorted and evaluated during the last 5 years.[3] Over three-fourth of the world populations relies on medicinal herbs and their extraction for dreaded diseases. From herbs the isolated, purified and characterized compounds in disparity, might drop their natural viscidity or fall short to perform in the same way as in the core matrix that the unique item of food.[4,5] Based on the Food and Agriculture Organization (FAO), over 50,000 herbs are used in the customary folk medicine all over the world.[6] The active principles are obtained from the whole plant or from various parts like root, leaf, stalk, howl, flower, rhizome and kernel etc. Over 30 percent of the plant

species, at single time or others were used for therapeutic reason. In the United States, approximately one fourth of the drugs are obtained from plant based, while in Ethiopia, and other African nations, the contribution is as much as 80%.[7] In 19thcentury, therapeutic herbs were the core basis of active principles competent of healing most human diseases. Therapeutic herbs keeps on to be the foundation of confirmed medicaments and of modern and innovative drugs.[8] In the early 20th century new development has come into sight, as a number of Botanists underway reviewing and identifying medicinal herbs on the foundation of their employ in tribal and rural areas. Hence, the monetary significance of medicinal herbs are distributed much more to countries like Ethiopia and other African countries than to rest of the planet.[2]

Cordia Africana Lam. (Amharic-wanza) (family: Boraginaceae) is native of Africa, which is widely distributed in Angola, Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, South Africa, Sudan, Tanzania, and Uganda. It is a medium-sized evergreen tree grows upto 4-15m height, greatly branched, umbrella-shaped circlet. Bole normally curved or crooked. Bark greyish-brown to dark brown, a multipurpose tree, providing food, medicines and materials for the neighbouring inhabitants. Habitually gathered from the wild, it is also cultivated for the timber, its edible berries, as shade tree in coffee plantations, as a medicinal plant.[9]

Leaves are alternate, simple, ovate around 15cm long with 3.5-10.2 cm broad. It is finely fibrous, dark green or paler emerald with well-known equivalent tertiary net-nerves. Apex are generally tapered and bottom curved. Flowers are gorgeous white mass with 1cm calyx and strappingly ridged, funnel-shaped lobes. Fruit are smooth, rounded fleshy with2 cm long. Fruits are green when young, which turns yellow to orange when mature. With Fruits contain 2-4 seeds without endosperm.

The *Cordia* genus contains about 250 species. The wood-ash combined with butter, is treated for certain skin diseases. Decoctions of leaves are infused to treat headache, nose bleeding, dizziness and vomiting during pregnancy. The leaves are desiccated and pulverized to spray over wounds. A root is used as a treatment for jaundice and schistosomiasis. The fresh juicy bark is utilized to tie a broken bone; this support is changed seldom with a fresh one until the bone is healed. Bark and fruits are prepared as stimulating tonic, used to treat fatigue and exhaustion while on a journey. Bark is used to treat many fungal disease.[10] In the preliminary phytochemical study, benzene, CCl_4, hexane and aqueous solvent extracts of leaves, stem and fruits of *C.africana* were investigated qualitatively using standard prescribed tests. Along with that *in vitro* antioxidant actions of plant extract was analyzed by measuring its total flavonoid, total phenol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing property.

MATERIAL AND METHODS

Chemicals

Ascorbic acid, Ferric chloride, HCl, Dragendorff's reagent, hexane, benzene, carbon tetrachloride, gallic acid, chloroform, H_2SO_4, Folin-Ciocalteu reagent, 1,1-diphenyl-1-picrylhydrazyl and glacial acetic acid, 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

Cordia africana Lam. was collected from Jimma University Garden, Jimma, South West Ethiopia in the month of October-2014. The plant has been taxonomically identified and authenticated by the Jimma University Botanist Dr. Florence and kept in Jimma University Botanical Science and Herbarium for future references.

Benzene Extract of Leaves, Fruit and Stem of *Cordia Africana*

The shadow dehydrated roughly powdered of leaves, fruits and stem of *C.africana* was engrossed and haul out with benzene for 72hrs. After finishing point, the defatted solutions were sieved by filter paper Whatman No.1 to eliminate

any contamination. The extract was intensed by vaccum desiccators to reduce the degree; the concentrated samples were relocated to another beaker and the residual solvent was further vaporized. Finally the dark greenish yellow coloured extract was formed and again it was kept in vaccum desiccators to get rid of unnecessary wetness. Dehydrated extract was stored in sealed container for Phytochemical screening studies.

Aqueous, CCl_4, and Hexane Extracts of Leaves, Fruit and Stem of *C.Africana*

The residues left after benzene extraction was dehydrated and then engrossed separately with aqueous, CCl_4 and hexane respectively up to 3days. After finishing point of extraction, the organic solvents were eliminated by vaccum desiccators. Dark greenish yellow colour extracts were formed and then stored in a sealed container for further studies.

Preliminary Phytochemical Studies[11,12]

The extracts obtained (benzene, methanol, carbon tetrachloride, and hexane) were employed to the subsequent phytochemical screening.

Test for Alkaloids

Dragendorff's test

Take 1ml of the solvent extract, add equal volume of distilled water followed by1ml of 2molar solution of HCl added until acidification reaction take place. To add this 1ml of Dragendorff's reagent. Orange or red colour is formed, indicated that the occurrence of alkaloids.

Hagger's Test

Take 1ml of the solvent extract in a cleaned test tube, add 1ml of Hager's reagent. Yellow precipitate is formed, indicated that the occurrence of alkaloids.

Wagner's Test

Take 1ml of solvent extract acidified with 1ml of 1.5 % v/v of HCl and add 1ml of Wagner's reagent. Formation of yellow or brown precipitate, which indicated that the occurrence of alkaloids.

Mayer's Test

Take 1ml of Mayer's reagent, add 1ml of solvent extract. White or pale yellow precipitate is formed, indicated that the occurrence of alkaloids.

Test for Carbohydrates

Anthrone Test

Take 1ml of solvent extract and 10ml of distilled water in a test tube, shaken vigorously and filtered. To this filtrate, add 1ml of anthrone reagent and mixed. Green or blue color is formed, indicated that the occurrence of carbohydrates.

Benedict's Test

Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, add 3ml of Benedict's reagent and kept in a boiling water bath for 5min. Formation of reddish brown colour indicated that the occurrence of reducing sugar.

Fehling's Test

Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, add 1ml of Fehling's solution A and 1ml of Fehling's solution B and kept in a boiling water bath for 5min. Formation of reddish brown colour indicated that the occurrence of reducing sugar.

Molisch's Test

Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, 1ml of Molisch reagent added followed by 2ml of Conc. H_2SO_4 added in the side of the test tube. Formation of two junction, which indicates the occurrence of carbohydrates.

Test for Flavonoids

Shinods Test

Take 1ml of solvent extract diluted with 3ml of ethanol followed by 2ml dilute HCl and pinch of Mg in a test tube, shaken gently. Appearance of pink or brown precipitate indicated that the occurrence of flavonoids.

With Con. H_2SO_4 Test

when treated with Con. H_2SO_4, appearance of the following colour like yellow colour (anthocyanins), yellow colour change to orange (flavones); orange colour change to crimson (flavonones) respectively.

Test for Glycosides

Molisch's Test

Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, 1ml of Molisch reagent added followed by 2ml of Conc. H_2SO_4 added in the side of the test tube. Formation of two junction, which indicates the occurrence of glycosides.

Test for Proteins and Free Amino Acids

Millions Test

Take 1ml of solvent extract with 1ml of Millions reagent, shake gently. Appearance of cherry red color indicated that the occurrence of free amino acid.

Ninhydrin Test

Take 1ml of solvent extract with 1ml of Ninhydrin reagent, shake gently. Formation of violet color indicated that the occurrence of free amino acids.

Biuret Test

Take 1ml of solvent extract with 1ml of 10% NaOH and 1ml of 1% copper sulphate in a test tube, shake gently. Development of purple color indicated that the occurrence of proteins.

Test for Gums and Mucilage

With 95% Alcohol

Take 1ml of solvent extract with 25 ml of 95% alcohol in a test tube, shake gently and filtered. The residue was air dried and examined for its bulging property. It indicated that the occurrence of gums and mucilages.

Test for Anthraquinones

Take 2ml of the solvent extracts acid hydrolysed with Conc. H_2SO_4 followed by extracted with benzene. Add 2ml of dilute ammonia. Appearance of rose pink color indicated that the occurrence of anthraquinones.

Test for Saponins

Foam Test

Take 5ml of solvent extracts in a test tubes add a drop of sodium bicarbonate, shaken vigorously and kept it stand for 3min. Development of cloudy white precipitate indicated that the occurrence of saponins.

Test for Sterols

Liebermann-Buchard's Test

Take1ml of solvent extract in a test tube and add acetic anhydride and kept in a boiling water bath for 5min, then cooled followed by 1ml of Con. H_2SO_4 added along the sides of the test tube. Appearance of green color indicated that the occurrence of steroids.

Salkowski Reaction

Add 1ml of solvent extract diluted with chloroform and followed by 1ml of Con. H_2SO_4added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of steroids.

Test for Fixed Oils

Spot Test

Take 0.5ml of solvent extract and pressed in between the two filter papers. Formation of oil stains on the paper indicated the existence of fixed oil.

Add 1ml of 0.5N alcoholic KOH and 1ml of solvent extract along with a single drop of phenolphthalein in a test tube. The residues were kept in a boiling water bath for 20min. Appearance of soap or incomplete neutralization of alkali indicated that the occurrence of fixed oils.

Test for Triterpenoids

Add 2ml of solvent extract and 1 ml of $CHCl_3$ followed by 1 ml of acetic anhydride in a test tube and shake gently. Add 1ml of Con. H_2SO_4added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of triterpenoids.

Test for Phenolic Compounds and Tannins

About 5ml of solvent extracts and equal volume of water added and perform the following reagent for confirmation of phenolic compounds and tannins.

Ferric Chloride Reagents

It gives a violet color

Gelatin Containing Sodium Chloride

It gives a white precipitate.

Lead Acetate Solution

It gives a white precipitate

***In Vitro* Antioxidant Assay**

Total Phenolic Content

The total phenolic content was estimated using the Folin–Colcalteu method, explained by Singleton et al.[13] with slight modification based on Liu.[14] In summary, 1ml of distilled water along with 125µl of the Folin–Colcalteu reagent were taken and add 125µl of plant extract. Then these samples were allowed to keep for 6 min in room temperature. Added 1.5 ml of 7% Na_2CO_3. Final volume was made to 3 ml using distilled water. Then it was kept in the room temperature for 90 min and the absorption measured at 760 nm against water as a blank.

The quantity of total phenolics was calculated as gallic acid equivalents (mg gallic acid/g plant extract) through the calibration curve of gallic acid.

Total Flavonoid Content

The total flavonoid contents were quantified using a spectrophotometric method depicted by Dewantoet al.[15]

Add 0.5 ml of plant extract, 75 µl of 7%$NaNO_2$ solution, 0.2ml of a 10% $AlCl_3$, 0.5 ml of 1M NaOH solution were taken. Final volume was made to 2.5 ml using distilled water.

Then it kept in the room temperature for 5 min and the absorption the absorption measured at 510 nm against the same mixture without plant extract, as a blank. The quantity of total flavonoids was calculated as mg Quercetin equivalent/g plant extract.

Reducing Power Assay

Reducing power was assayed by the method of Oyaizu[16] with minor changes. In summary, Added different concentrations (10-160µg/ml)of the plant extracts and 500 µl of 0.2 M phosphate buffer (pH 6.6), 500 µl of 1% potassium ferricyanide in a test tube and kept for water bath incubation at 50^0C for 20 min. Then the reaction was terminated by using 10% tri chloroacetic acid followed by centrifugation at 3000 rpm for 10 min. Take 500µl of supernatant mixed with 500 µl of distilled water followed by 100 µl of 0.1% ferric chloride, and the absorption measured at 700 nm against water as a blank.

Free Radical Scavenging

It was determined by DPPH method. The radical scavenging potential of the plant extracts were determined by the method of Brand-Williams et al.[17] with minor changes. In summary, 2ml of the plant extracts diluted with ethanol, followed by 2ml of an ethanol solution of DPPH (0.0025 g/100 ml) and the mixture kept for 20 min at room temperature, and the absorption measured at 517 nm against water as a blank.

Statistical Analysis

All values are expressed as mean ± SD of triplicate results.

RESULTS AND DISCUSSION

Phytochemical Screening

Table 1 shows the phytochemical constituents of *C.africana* leaf extract which revealed the presence of alkaloids, protein and aminoacids, total phenol and tannins, and flavonois in the above extract.

In Vitro Antioxidant Properties

Table 2 gives the antioxidant property results of the aqueous-methanolic extract of *C.africana* leaf. Total phenolic content was 224.78mg gallic acid equivalent/g extract while the total flavonoid was 335.45mg equivalent/g Quercetin equivalent/g extract.

Table 1: Phytochemical investigation of leaves, stem and fruits of *Cordia africana* Lam using aqueous, benzene, CCl_4 and hexane solvents

Analysis	***Cordia africana***											
	Leaves				**Stem**				**Fruits**			
	Benzene	**CCl_4**	**Hexane**	**Aqueous**	**Benzene**	**CCl_4**	**Hexane**	**Aqueous**	**Benzene**	**CCl_4**	**Hexane**	**Aqueous**
Alkaloids	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Protein and aminoacids	-	+++	-	-	-	-	-	-	++	+	-	-
Anthra-quinones	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	++	++	++	++	++	++	++	++	++	++	++	++
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	+	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	+
Total phenols and Tannins	++	++	++	++	++	++	++	++	++	+++	++	++
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	-

+++ = 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

Table 2: In vitro antioxidant indices of leaves extract of C.Africana

Metabolites	**Level**
Total Phenol	224.78mg Gallic acid equivalent/g extract
Total flavonoid	335.45mg Quercetin equivalent/g extract

Ferric reducing property of leaves extracts of *C.africana* illustrated in Figure 1. The results exhibited that the leaves extracts of *C.africana* note worthy high reducing potential when compared with vitamin C. The experiential effects were dose dependent manner with the highest activity occurring at the 160µg/ml concentration of the plant extract.

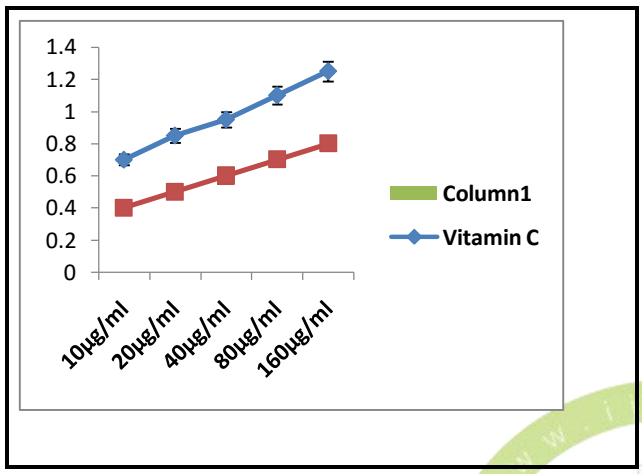

Figure 1: Ferric reducing properties of leaves extracts of *C.africana*. values are expressed as mean ± SD (n=3)

DPPH radical scavenging activity of *C.africana* leaf extracts illustrated in Figure 2. The results exhibited that the leaves extracts of *C.africana* note worthy strong radical scavenging activity against DPPH radical. The experiential effects were dose dependent manner with the highest activity occurring at the 160µg/ml concentration of the plant extract.

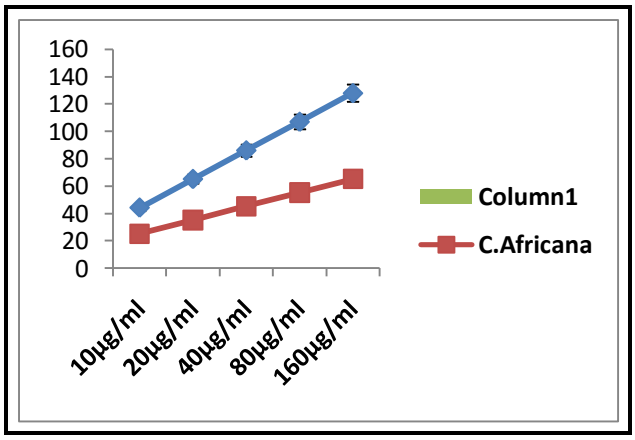

Figure 2: DPPH radical scavenging activity of leaves extracts of *C.africana*. values are expressed as mean ± SD (n=3)

DISCUSSION

In advanced years, the finding of edible plants with antioxidant properties have been the heart of the probe. Based on most of the investigation on medicinal herbs, It is alleged that medicinal plants can avert or defends tissues against free radical damage.[18] Free radicals such as RNS and ROS creates number of a variety of metabolic diseases in human.[19] The advantage of therapeutic effects of plant sources usually result from the complex of secondary metabolites present in the herbs, by additive or synergistic action of numerous dynamic composites per forming as one or manifold target places connected with a physiological progression.[20] The note worthy huge amount of total phenols and flavonoids (144.18mg gallic acid equivalent/g extract and 256.858mg Quercetin equivalent/g extract respectively) may be evocative of significant antioxidant potentials. Total phenolic ingredients are groups of antioxidant elements which plays as free radical scavengers.[21] The chief vigorous neutraceutical compounds in medicinal herbs are flavonoids. It is distinguished as a term neutraceutical, which are safe food additives that has been systematically confirmed health benefits for equally the healing and anticipation of the disease.[22]

Another distinguished property of almost every group of flavonoids is their capacity to acts as antioxidants. As is typical for phenolic compound, it can play as effective antioxidants and metal chelators.[23] Flavonoids are well known antioxidant and its effects on human diet and health care are noticeable. It has been recognized to have potential antioxidant, antibacterial, antihyperglycemic, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antimutagenic, antiviral, and anticarcinogenic activities due to presence of hydroxyl functional groups, which employ of scavenging or chelating process.[24-26]

The presence of tannins and alkaloids in *C.africana* is indicative of its efficient bioactivity. The tannins in the plant demonstrated that the plant plays a vital role as antidiarrhoea

and antihaemorrhagic agent.[27] The representation of scavenging DPPH radical and assessment of reducing power is a extensively used technique to assess the free radical scavenging capacity of various samples.[28] Based on the results, the plant has potent radical- scavenging activities and hence antioxidant activity of all the extracts increased with increasing concentration.

CONCLUSION

The present study showed that the benzene, CCl_4, hexane and aqueous extracts of *C.africana* leaves, stem and fruit extracts exhibited noteworthy total flavonoid and phenolic content and also showed a potent free radical scavenging activity. The plant has demonstrated as basis of antioxidant additives.

ACKNOWLEDGMENT

We sincerely acknowledge to the Staff of the Biomedical Sciences, Jimma University, Jimma, Ethiopia who really gave support and cooperation in the study.

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