Evaluation of Antibacterial, Antioxidant and Phytochemical screening of *Chamaecyparis obtusa* (Crippsii) Fruits

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Abstract

This present study was carried out in Khartoum State-Sudan, during October (2019). The species *Chamaecyparis obtusa* (Crippsii) belong to family Cupressaceae, locally known as (Shagarat Bakhor Alanfar) it was chosen because it's using traditionally in treatment of many abdominal disease. Phytochemical constituents were investigated to detect the effects of antibacterial and antioxidant activities. The dried fruits of *Chamaecyparis obtusa* was extracted successively with (ethanol), The phytochemical screening carried out on ethanolic extract of species fruits and includes, (alkaloids, flavonoids, sterols, tannins, triterpenes, cardiac glycosides, phenols and absent of saponins). The antibacterial activity was investigated against two standard bacteria (Gram positive; *Staphylococcus aureus*) and (Gram negative; *Escherichia coli*,), the results of antibacterial activity of extract at different concentrations and inhibition zone were measured in (mm) and the range of inhibition zone was found between 15-22mm.Ethanolic extract of *Chamaecyparis obtusa* fruit reflected the strongest antioxidant activity (81 ± 0.04) as compared with the positive control.

Keywords: *Chamaecyparis obtusa*; Crippsii; Folk medicine; Gabal Maraha-area; Medicinal plants.

{**Citation:** Yasmin Hassan Elshiekh, Mahdi Abdelmageed Mohammed Ali, Rahma Abubakr Musa, Yagoub Abdella Ali. Evaluation of Antibacterial, Antioxidant and Phytochemical screening of *Chamaecyparis obtusa* (Crippsii) Fruits. American Journal of Research communication, 2020, Vol8(9): 15-24} www.usa-journals.com, ISSN: 2325-4076.

1. Introduction

Since the ancient times, extracts of many plants were used in ethno-pharmacy and have been of vital importance in the treatment of many diseases. The activity of these extracts in the therapeutic sense depended upon the chemical composition of the used plant species. Since at that time it was not much known about the chemical composition of plants, the emphasis was placed on its discovery. In the last decades, it has been established that plants have a large number of secondary metabolites as alkaloids, terpenoids, phenolic acids, flavonoids, tannins, lignins, quinones, coumarins and other that are responsible for broad spectrum of biological activities [1, 2, 3, and 4].The Sudanese medicine plants represents many unique blend of indigenous cultures with others countries such as Egyptian, Indian, Arabian, East and West African cultures [5]. Most of Sudanese people use plants at the main traditional medicinal source to treat infectious diseases [6]; the medicinal plants have played an important role in the treatment of diseases especially in rural areas [7]. The medicinal plants contain a number of secondary metabolise constituents such as alkaloids, flavonoids, tannins, saponins, glycosides and others isolated and used as an important source of indispensable drugs [8]. State that, medicinal plants are known by their required clinical effects on the abnormal living tissues or organs while toxic ones are known by their ability to cause a non-required physiological deviation in animals' bodies, the traditional medicinal plants are increase in both developing and industrialized countries.[9,10]; reported that both literate and illiterate people still use local plants as drugs in many conditions [11,12], cited that many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labor cost and selection of the superior plant stock and over exploitation by pharmaceutical industry [13].

2. Materials and Methods:

In this study the chemicals and reagents used were analytical grade such as ethanol, acetic anhydride, sulphuric acid, gelatine salt, ferric chloride, reagents (Wagner, Hager, and Dragendorffs), aluminium chloride and potassium hydroxide.

2.1. Plant material, collection and identification

Chamaecyparis obtusa fruits were collected in March 2019 from Gabalmarah , South Darfur State- Sudan, and identified in herbarium of natural research Centre and compared with herbarium of Faculty of Science University of Bahri. .

2.2. Preparation of Crude Extracts:

"100g of the dried fruits were weighted and extracted successively with ethanol",(300ml absolute 80%) by shaker apparatus for 18 hours at room temperature", then was filtrated through Whitman No 1 filter paper and dried after extraction, followed by concentrated under vacuum room. The crude extracts were kept in dark bottle at 20ºC.

2.3. Phytochemical screening of ethanol extracts of the plant:

Phytochemical screening for the active constituents was carried out for extract using the methods carried by [14, 15].And the extract using the methods described by [16]. The detection tests of (alkaloids, flavonoids, sterols and Triterpenes, tannins, saponins, phenols and cardiac glycosides) were carried out.

2.4. Biological Activities

2.4.1. Preparation of media.

"28g of powdered nutrient agar was weighted, dispersed in 1 liter of distilled water and allowed to soak for 10 minutes, "swirl to mix then sterilized by autoclaving for 15 minute at 121c, cooled to 47 ºC", mixed well then poured into Petri dishes.

2.4.2. Testing of bacteria organisms

One gram negative and one gram positive of bacteria were tested as bellow:

Staphylococcus aureus (ATCC 25923 Gram positive bacteria), *Escherichia coli* (ATCC 25922 Gram negative bacteria).

One ml of the standardized bacterial stock suspension 108-109 C.F.U/ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45ºC0. 20ml aliquots of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates 5 cups (10mm in diameter) were cut using a sterile cork borer (No.5) and agar disk were removed .Alternate cups were filled with 0.1ml sample of each of the extract dilution in ethanol using automatic micro-liter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Three replicates were carried out for each extract against each of the test organism. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and mean values were tabulated [17].

2.5. DPPH (2,2-diphenyl-1-picrylhydrazyl):

DPPH radical scavenging was determined according to the methods of[18].With some modification. In 96-wells plate the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 ºC. The concentration of DPPH was kept at (300µM) the test sample were dissolved in DMSO while DPPH was prepared in ethanol after incubation , decrease in absorbance was measured at 517nm using multi-plate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

3. Results:

3.1. Phytochemical screening of *Chamaecyparis obtusa* fruits

Ethanol solvent was used in successive polarities to extract secondary metabolites from *Chamaecyparis obtusa* fruit and their properties was cited in table (1) which was reported that, the result of *Chamaecyparis obtusa* fruit contain amount of secondary metabolites (alkaloids, flavonoids, triterpenes, sterols ,phenols, tannins, cardiac glycosides) and absent of saponins.

Table (1): Phytochemical screening of *Chamaecyparis obtusa* fruits

Secondary metabolites	Reagents	Results
Alkaloids Acidic	Dragendroff's	+
	Wagner's	-
	Hager's	+
Flavonoids	Ammonia 1%	+
	Na OH	+
	Mg \H2SO4	+
Tannins	Fe Cl3 5%	+
	Lead acetate	+
Sterols	Salkowski	+
	Liebermann's	+
Triterpenes	Salkowski	+
	Liebermann's	+
Saponins	Foam test	-
Cardiac glycosides	Keller-Killiani	+
Phenols	Ellagic acid test	+

Key: present = (+) and absent= (-).

3.2. Antibacterial activity of *Chamaecyparis obtusa* fruits:

Result of antibacterial activity of ethanolic extract of *Chamaecyparis obtusa* fruits at concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml), were showed that there were inhibition zone in the cup plate agar diffusion of ethanol against two bacterial strains and it measured in (mm) ,the range of inhibition was found 15-22mm in (*E.coli*) and 21-16mm in (*Staphylococcus aureus*).

Table (2) Antibacterial activity of *Chamaecyparis obtusa* fruits at concentrations 100, 50, 25 and 12.5 mg/ml.

Plant extract	Concentrations in mg/ml	Zone of inhibition in diameters (mm)	
		Staphylococcus aureus	*Escherichia coli*
Ethanol	100	21	22
	50	19	18
	25	18	16
	12.5	16	15

3.3. Antioxidant activity of *Chamaecyparis obtusa* fruits:

Table (3) Antioxidant result

No	Sample	% RSA ± SD (DPPH)
1	*Chamaecyparis obtusa*	81 ± 0.04
Standard	Propyl Gallate	91 ± 0.01

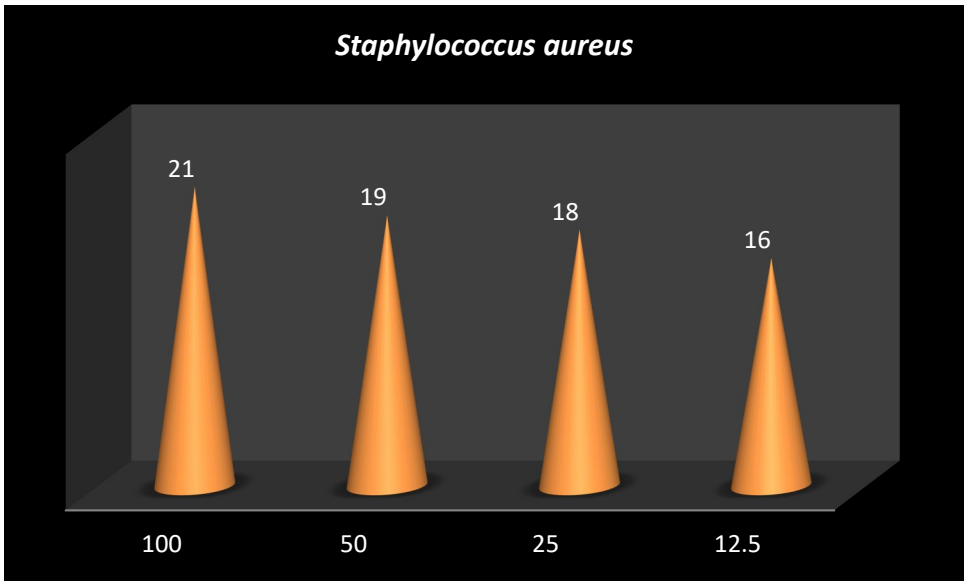

Figure 1: Antibacterial activity of *Chamaecyparis obtusa* against *Staphylococcus aureus* at Concentrations(100, 5, 25, and 12.5).

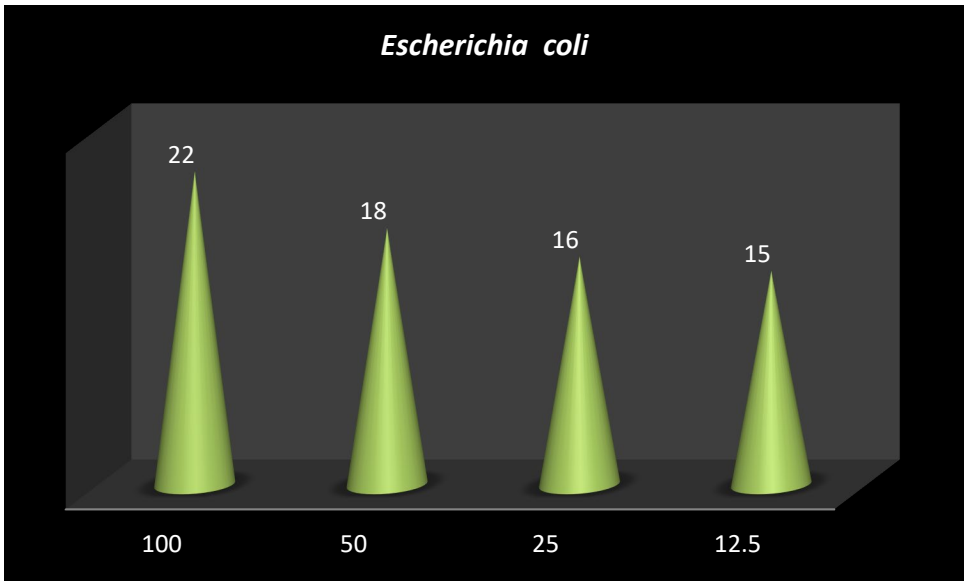

Figure 2: Antibacterial activity of *Chamaecyparis obtusa against Escherichia coli* at Concentrations (100, 5, 25, and 12.5).

4. Discussion:

Phytochemical screening of *Chamaecyparis obtusa* fruit showed the presence of alkaloids, flavonoids, triterpenes, Sterols ,phenols, tannins cardiac glycosides and absent of saponins, in antibacterial activity the ethanol extract showed high activity at all concentrations (100,50,25,12.5) against *Staphylococcus aureus.* (21,19,18,16), high activity against *Escherichia coli* (22,18,16,15).These may lead to use this species as medicinal plant for many antibacterial drugs. Free radicals and its adverse effects were revealed in the last decade [19]. Extreme addition of free radicals results in oxidative stress. This is one of the main causes of creation and progress of diseases and early aging [20]. Antioxidants are substances capable to get rid of free radicals and prevent its harm [21, 22]. Flavonoids, phenols, tannins and alkaloids are active ingredients in medicinal plant that are related to antioxidant activities [23]. The result proves that, *Chamaecyparis obtusa* is an excellent source of natural antioxidants that can be used to decrease the effect of oxidative damage.

Conclusion:

This study serves customs in developing countries in addition to contributing further depths to the growing literature on plant materials recognized as a reservoir of important to antibacterial and anti-oxidant compounds. The findings in this study have hence provided scientific support for the ethno medical antibacterial activity of extracts of the *Chamaecyparis obtusa* fruits. The phytochemistry of the plant shows that the extract contains secondary metabolites. Hence the constituent with the antibacterial and anti-oxidant activities can be reported to be from the above phyto-constituents.

Acknowledgements

The authors are very grateful to people and laboratories for sharing their knowledge on traditional herbal medicine.

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