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RESEARCH ARTICLE

Ethanolic Extraction, Phytochemical Screening, Antimicrobial and Antioxidant activities of *Chamaecyparis obtuse* Fruits .

Dr. Mahdi Abd elmageed Mohammed Ali.[1*] | Dr. Rahma Abubaker Musa[1]

[1]Department of Biology &Technology, College of Applied Science & Industrial, University of Bahri. Khartoum

Abstract

This 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) was chosen because it's using traditionally in treatment of many abdominal disease. Phytochemical activities were investigated to detect the effects of antimicrobial and antioxidant; this plant was collected from Gabal maraha , South Darfur State, western Sudan. The dried fruits of *Chamaecyparis Obtusa*was extracted successively with (ethanol), The phytochemical screening carried out on ethanolic extract of species fruits and showed as table: (1) , that there were secondary metabolize (alkaloids ,flavonoids, sterol, tannins , triturpenes ,cardiac glycoside, phenol and absent of saponnins). The antimicrobial activity of extract were evaluated against two standard bacteria (Gram positive; *Staphylococcus aureus*) and (Gram negative; *Escherichia coli*,), the results of antimicrobial activities of extract at different concentrations and inhibition zone were measured in (mm) table (2) the range of inhibition was found between 15-22mm .Antioxidant was tested and The result of it cited that there is high antioxidant activity in the extraction table (3).
Keywords: Folk medicine, Gabal Maraha area, Medicinal plants, phytochemical screening, antioxidant.

1 | INTRODUCTION

The Sudanese medicine plant represents many unique blend of indigenous cultures with others countries such as Egyptian, Indian, Arabian, East and West African cultures. Gamal, E. D ,*at el*. (1997)". Most of Sudanese people use plants at the main traditional medicinal source to treat infectious diseases. Hatil hashim , *at*,. *el*. (2009); the medicinal plants have played an important role in the treatment of diseases especially in rural areas. Elgazali B. *at* ,*el*,. (1994)". 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. Wondergen, *at*.,*el*. (1989)". 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. WHO. (1998) Koko, *at* ,*el*.(1976); reported that both literate and illiterate people still use local plants as drugs in many conditions Gibbons, S. (2008) V.L. Williamsa *at*, *el*.(2011), ted 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. Holm LG, *at*., *el*, (1977)".

2 | OBJECTIVE:

The objective of this study is to determine the extraction, phytochemical screening and antimicrobial and antioxidant activities of *Chamaecyparis Obtusa* fruits.

3 | MATERIAL AND METHOD:

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.

3 .1. Plant material, collection and identification:

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

Supplementary information The online version of this article ( ) contains supplementary material, which is avail-able to authorized users.

Corresponding Author: *Dr. Mahdi Abd elmageed Mohammed Ali.*
Department of Biology &Technology, College of Applied Science & Industrial, University of Bahri. Khartoum

3.2. 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. .

3.3. Phytochemical screening of ethanol extracts of the plant:

Phytochemical screening for the active constituents was carried out for extract using the methods carried by. Shelley, B. C. (2009)., Akinyemi, K. O., *at*, *el*. (2005),.And the extract using the methods described by. Martinez A , *at*,. *el*, (2003), Kavanagh, F. (1972).The detection tests of (alkaloids, flavonoid, Triterpenes and sterols, Tannins, Saponins, phenol and Glycosides) were carried out. .

3.4. 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.

3.4.1. Testing of bacteria organisms

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

Staphylococcusaureus (ATCC 6538 Gram +ve Bacteria).; *Bacillus subtilis*

(ATCC6633 Gram -ve 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 45 ºC 0. 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 microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC 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.

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

DPPH radical scavenging was determined according to the methods of. Shimada K, *at*,. *el*. (1992) .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 multiplate 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.

4 | RESULT:

4.1. Phytochemical screening activity of *ChamaecyparisObtusa* fruit

Ethanol solvent was used in successive polarities to extract secondary metabolites from *ChamaecyparisObtusa* fruit and their properties was cited in table (1) which was reported that, the result of extractives values of *Chamaecyparis Obtusa* fruit contain amount of secondary metabolize (alkaloids, flavonoids, triterpenes, Sterols, Triterpenes ,phenol, cardiac glycosides) and absents of saponins.

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

TABLE 1: Phytochemical screening of Chamaecyparis Obtusa fruit

Successive method of extraction	Test	Secondary metabolite
Ethanol		
+	Dragendroff's	Alkaloids
-	Wagner's	
+	Hager's	
+	Ammonia 1%	Flavonoids
+	Na OH	
+	Mg \H2SO4	
+	Fe Cl3 5%	Tannins
+	Lead acetate	
+	Salkowski	Sterols
+	Liebermann's	
+	Salkowski	Triterpenes
+	Liebermann's	
-	Foam test	Saponins
+	Keller-Killiani	Cardiac glycosides
+	Ellagic acid test	Phenol

4.1.2. Antimicrobial activity of *Chamaecyparis Obtusa* fruit:

Result of antimicrobial activates of ethanolic extraction 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: Antimicrobial activities of Chamaecyparis Obtusa fruit at concentration 100mg/ ml.

Zone of inhibition in diameters (mm)		Concentration in mg/ml
E.coli	Staphylococcus aureus.	
22	21	100
18	19	50
16	18	25
15	16	12.5

4.1.3. Antioxidant activity of *Chamaecyparis Obtusa* fruit:

Ethanol extract was showed high antioxidant activity as table below:

TABLE 3: Antioxidant result:

NO	Sample code	% RSA ± SD (DPPH)
1	C.O= Chamaecyparis Obtusa	81 ± 0.04
Standard	Propyl Gallate	91 ± 0.01

5 | DISCUSSION:

.1. Phytochemical screening of *Chamaecyparis Obtus a*

The phytochemical screening were carried out on different extracts of *Chamaecyparis Obtusa* fruit and it showed to contain amount of secondary metabolize and absent of saponins,

5.2. Antimicrobial activities of *Chamaecyparis Obtus a* :

The ethanol extract showed high activity at all concentrations (100,50,25,12.5) against *Staphylococcus aureus.* (21,19,18,16),high activity against *E.c* (22,18,16,15). These may lead to use this species as medicinal plant for many anti-microbial drugs.

5.3. Antioxidant activities of *Chamaecyparis Obtusa*:

Ethanol extract was showed high antioxidant activity; it gave good result against two tested microorganisms and very good result about antioxidant.

6 | 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 antimicrobial and anti-oxidant compounds. The findings in this study have hence provided scientific support for the ethno medical antimicrobial activity of extracts of the *Chamaecyparis Obtusa* fruits. The phytochemistry of the plant shows that the extract contain secondary metabolise . Hence the constituent with the anti-microbial and anti-oxidant activity can be reported to be from the above phyto-constituents.

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7 | REFERENCE:

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