Ahmed A. Motar et al /J. Pharm. Sci. & Res. Vol. 10(4), 2018, 693-696

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Qualitative and quantitative study of active compounds of *Casuarina cunninghamiana L.* parts

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Abstract

Objective: The objective of study prepare crude extract and phenolic compound of *Casuarina cunninghamiana* leave, fruit and bark .
Methods: collect and prepare sample ,phytochemicals ,TLC Technique , FT-IR Technique , HPLC .
Results: The prepared phenol were identified through the use of some chemical tests such as thin layer chromatography (TLC) reached to five compounds ,seven compounds in fruit and four compounds in bark. Four chemical active groups includes (OH , CH , C=O , C=N) were identified using Fourier transform infrared spectrometry (FT-IR) .High performance liquid chromatogram (HPLC) this technique isolated one phenol (gallic acid) and three flavonoids (kampherol , quarcetin, rutin) from leaves, fruit and bark .

Key words: *Casuarina cunninghamiana* L,, .crude extract , phytochemical, TLC, FTIR and HPLC.

INTRODUCTION

Throughout ages of human existence, they used to familiarize themselves plants and used them in different ways. First, by searching for food and successfully deal with human sufferings began to distinguish plants which are suitable for medicinal purpose and pharmacological action. The relationship between plants and human have grown and increase with increases number of plants that used as medicines .The growth of knowledge to treat diseases continues to accelerate by increasing number of drugs originated from plant [1]. . The fact that medicinal plants is a widespread result from that they are healthier and safer than synthetic medicine [2]. Casuarinas are rich in phytochemical which present in various parts of this forest crop and have pharmacologically active [3]. Extraction of Casuarinas parts for the phytochemical screening is based on the solvent, solvents play a major role in the analysis of the phytochemicals present in the extract, because it has great impact in rate of extraction, compounds to be extracted, toxicity of the solvent could also affect the bioassay process [4].Some phenolic compounds in *Casuarina* are synthesized and accumulate in plant cells as a defense mechanism after exposure to microorganism [5]..Phenolic compounds such as hydroxyl cinnamic acids play important roles in lignification and therefore, influence the physical structure of plants [6].

Correlation between ingesting phenolic compounds and improved health has been reported in epidemiological studies [7]. The major phenol compounds that were isolated from leaves of *Casuarina equisetifolia* were gallic, protocatioic, chlorogenic, phenol.hydroxy benzoie, phenol.coumaric, syringic, vanillic and salicylic acid [8]. The plant is a source of biologically active compounds such as catechin , ellagic acid, gallic acid, quercetin and lupeol, which are antioxidants [5].

MATERIALS AND METHODS

Preparation of Plants Extracts

The plants were extracted with three types of solvents, which were hot distilled water methanol and ethanol . In both cases, the extracted three parts leaves ,fruits and bark of *Casuarina cunninghamiana* [9,10] .

preparation of phenol

The preparation of phenol from leaves, fruit and bark according to [9] .

Phytochemical screening

Chemical detection of the active components in plant extracts [7, 9].

preparation purification and Identification of phenolic compounds

To determine the purity and Identification of phenolic compounds according to thin layer chromatography(TLC) ,[9] , FT- IR spectra and HPLC technique

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening was done using color forming and precipitating chemical reagents on the three parts of casuarinas leaves , fruit and bark for hot aqueous extracts and alcohol (methanol and ethanol) which contain alkaloids phenolic , flavonoids compounds, saponins fig. (1) . Glycosides and tannins were found to be positive results according to Kishore and Rumana[20]

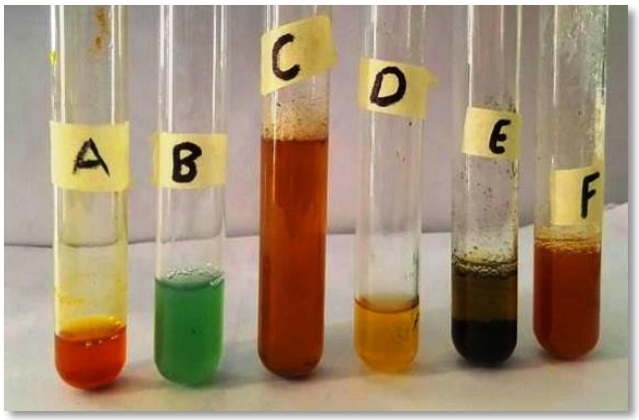

Figure (1) The results of phytochemical screening:
A- Alkaloid , B-Glycoside C- Saponins D- Flavonoid E- Phenolic F- Tannins

Identification of phenolic compounds

Thin Layer Chromatography

The analysis of TLC chromatography of phenolic compounds Fig. (2) shows the presence of several spots corresponding to solvent system n-butanol: acetic acid: water (4: 1: 5) (L: leaves , F: fruit , B : bark) Compound No. 1,2,3,4 ,5 were present in leave which extend R*f* from 28 to about 96 that explain number and properties compound on TLC plates and reflection factor for each compound(R*f*), the color of compound in visible light extend from light yellow to deep yellow while in Uv light extend from purple to pink compared with fruit present founded 7 compound No. 6, 7, 8, 9, 10,11, 12 which extend R*f* from 14 to about 98 , in visible light the color of compound extend from brown to deep yellow while in Uv light extend from brown to blue and bright pink . While in bark presented 4compound No. 13, 14, 15, 16 which R *f* extend from 40 to about 98, in Uv light extend from

brown to bright pink but in visible light extend from yellow – green to about yellow .The difference in the value of R*f* of studied phenols is credited to the degree of polarity and function groups of each compound and to the mobile phase, small R*f* value indicated for the low solubility of compound in the mobile phase therefore the compound slowly to move up, Large R*f* value indicated for the high solubility of compound in the mobile phase therefore the compounds readily moves up [11].

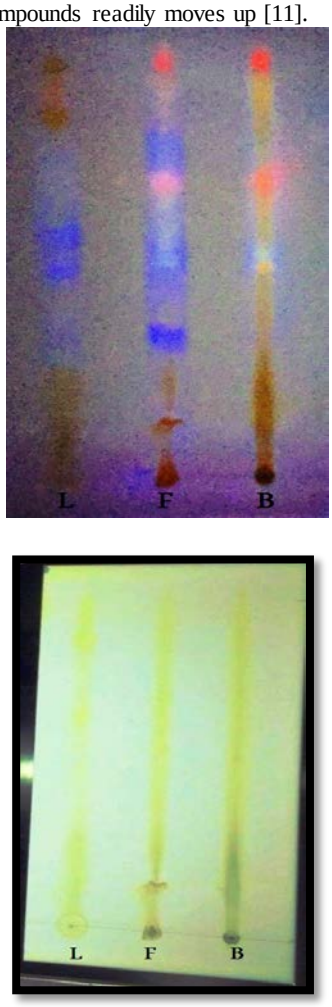

Figure 2: Thin layer chromatography for phenol extracts , system n-butanol: acetic acid: water (4: 1: 5) (L: leaves , F: fruit , B : bark).

The compound No. 2,4 in leaves extract this may be refer to gallic acid, isoliquiritigenin according to [9] which resemble by R*f* and color in Uv light . Leaves compound No. 3 resemble to compound No. 8 in fruit while compound No. 10 and 12 in fruit resemble to compound No. 15 and 16 in bark in R*f* and color in visible and Uv light . [12] Researcher study *Casuarina equistifolia* and present some compound resemble with my results but other compound not compatible , leaves compound No. 1 , 3 refer to tannin but No. 5 is unknown . In the bark No. 16 also refer to tannin .Compound No. 10 in fruit and compound No.15 in bark were compatible with R*f* 78 , but differ to other compound [13].

FTIR Spectra

The FTIR spectrum was used to identify the functional group of the active compound based on the peak value in the region of infrared radiation. The IR spectra of the studied phenol as KBr discs and of their representative spectra are shown in tables (2) and figures (3, 4) and (5).

The spectrum of leaves is characterized by three bands corresponding to the stretching vibrations of the OH (3408.22 **,** 3392.79) cm-1, CH aromatic (2920.23) cm-1 and C≡O (1708.93 **,** 1728.22) cm-1 groups . The absorption data of IR of fruit showed five stretching vibration bands, which confirmed the correctness of the proposed structure. These bands are OH, CH aromatic, CH aliphatic, C≡O and C=N groups which occur in (3375.43), (3147.83**,** 3049.46 **,** 3012.81), (2926.01**,** 2854.65) , (1743.65) , (1406.11) cm-1 respectively. While the spectrum of bark is represent by four bands OH (3412.08) cm-1, CH aromatic (2924.09) cm-1 , C≡O (1732.08 , 1714.72) cm-1and C=N (1462.04) cm-1. Leaves extracts of *C . equisetifolia* which founded OH , CH , and C=O group compatible with [14].

FT-IR analysis of the extracts shows a strong presence of hydroxyl group which is common in all phenolic compounds. The absorption bands were attributed to (OH) stretching vibrations from phenols, a group of compounds (chemical) containing hydroxyl functional groups (-OH) attached to an aromatic hydrocarbon [15]. [16] study phenolic compound in *Origanum vulgare seeds* founded active compounds C-H aliphatic , C=O group and C-H aromatic.

Medicinal plant *Aerva lanata* study active group in phenolic extracts found many active group resemble with my results in C-H stretch (carbonyl), C=O stretching (carbonyl) , C-N Stretching , O-H Stretching [17].

Table (1): Phenol compounds in *C. cunninghamiana* Leaves , fruit and bark by Thin Layer Chromatography (solvent system BAW: 4:1:5).

N0.	Plant Parts	R*f*	Colors in visible light	Color in UV light
1	Leave	28	Light yellow	Purple
2	Leave	53	Whit to yellow	Deep blue
3	Leave	56	Deep Yellow	Deep blue
4	Leave	89	Deep Yellow	Brown
5	Leave	96	Deep yellow	Pink
6	Fruit	14	Brown	Brown
7	Fruit	33	Deep yellow	Deep blue
8	Fruit	56	Deep yellow	Bright blue
9	Fruit	59	Deep yellow	Bright blue
10	Fruit	78	yellow	Light pink
11	Fruit	81	Deep yellow	Deep blue
12	Fruit	98	Deep yellow	Bright pink
13	Bark	40	Yellow to green	Brown
14	Bark	56	Yellow	White to pink
15	Bark	78	Yellow	Pink
16	Bark	98	Yellow	Bright pink

Table (2) : FT-IR spectral data of penolic compounds recorded as KBr discs (cm-1).

NO.	Compounds	OH Str.	CH Aromatic Str.	CH Aliphatic Str.	C=O Str.	C=N Str.
1	Leaves	3408.22, 3392.79	-	2920.23	1708.93, 1728.22	-
2	Fruit	3375.43	3147.83, 3049.46, 3012.81	2926.01, 2854.65	1743.65	1406.11
3	Bark	3412.08	-	2924.09	1732.08, 1714.72	1462.04

Str. = stretching

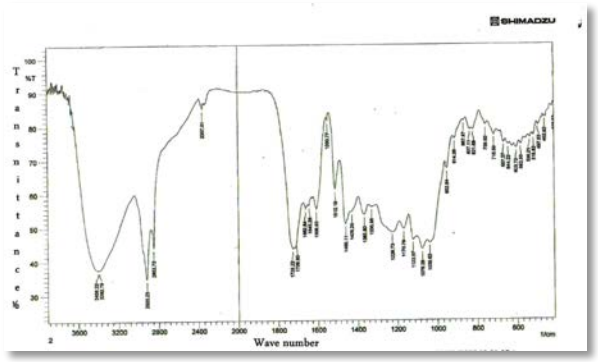

Figure (3): FT-IR in leaves

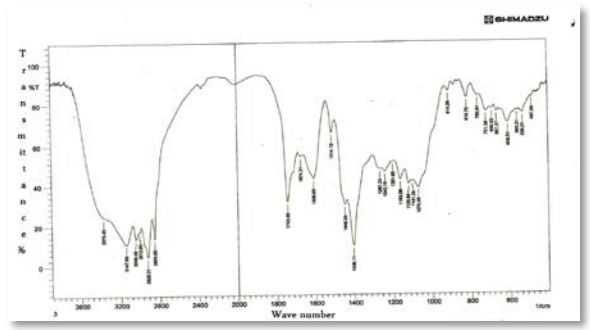

Figure (4) : FT-IR in fruit

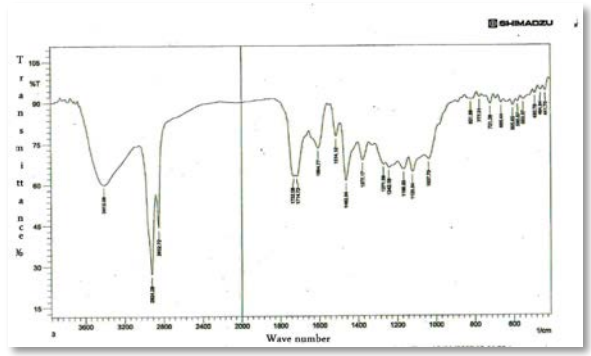

Figure (5) : FT-IR in bark

HPLC Technique

HPLC analysis of leaves , fruit and bark were done by shimadzu HPLC with flow rat 1ml / min , wave length 240 nm ,mobile phase : MeOH : D.W: A.A (60 : 35 :5) and stationary phase were octodial saline C18 (ODSC18) . HPLC analysis successfully provided the presence of 1 phenolic compound, retention time was : 3.077 min as compound gallic acid and 3 flavonoid , qurcetin 4.697 min, rutin 3.236 min and kampherol 7.445 min figure (6,7,8,9,10,11 and12) , table (3) .

Compounds were isolated from leaves which have the highest concentration was gallic acid 211.4 µg/ml followed by kampherol, rutin and qurcetin, 63.9, 50.3, 40.9 µg/ml, respectively . In fruit the highest concentration kampherol 210.1 µg/ml but the gallic acid had lowest concentration 36.4, the highest concentrated compound was

quarcetin 189.7 µg/ml in bark but kampherol had the lowest concentration 41.8 µg/ml . Previous studies such as [18] ,[4] and [8] compatible with our result in leave but in different concentrations , as well [19] and [20] mentioned that the methanolic extract of leaves contained kampherol ,qurcetin and rutin in *Casuarina species* .

Table (3) HPLC results of phenolic chemical composition and concentration of *C. cunninghamiana* for leaves , fruit , bark .

Stander	Retention time min	Concentration in leaves mg/ml	Concentration in fruit mg/ml	Concentration in bark mg/ml
Gallic acid	3.077-1.954	211.4	36.4	161.3
Kampherol	7.445	63.9	210.1	41.8
Qurcetin	4.697	40.9	36.9	189.7
Rutin	3.236	55.3	40.2	60.4

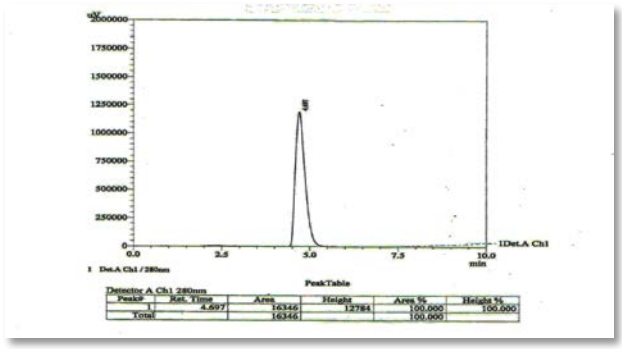

Figure (6) : HPLC of stander curve of Qurcetin

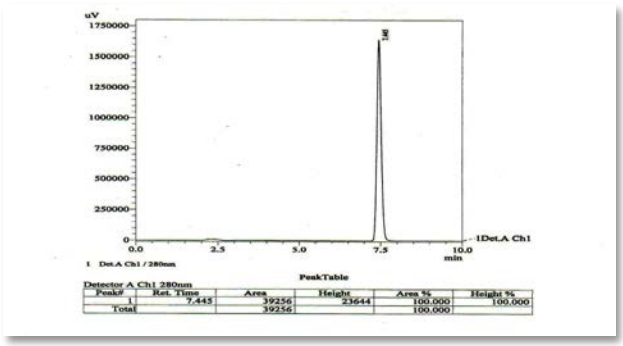

Figure (7) : HPLC of stander curve of Kampherol

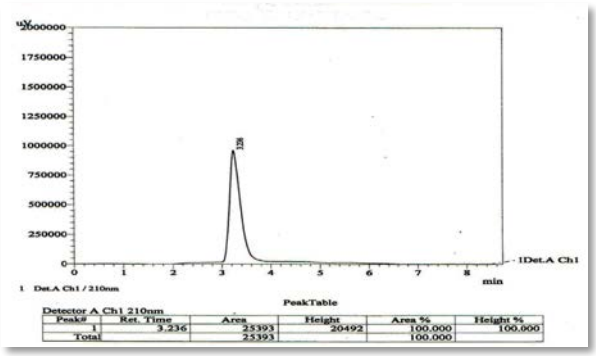

Figure (8): HPLC of stander curve of Gallic acid

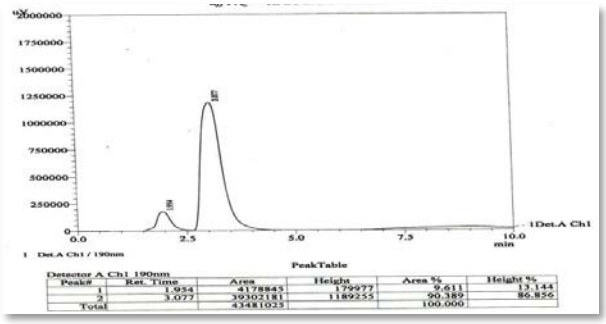

Figure (9): HPLC of stander curve of Rutin

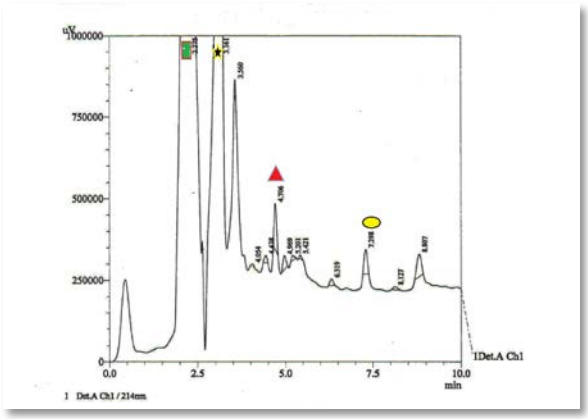

Figure (10) : HPLC curve of leaves extract

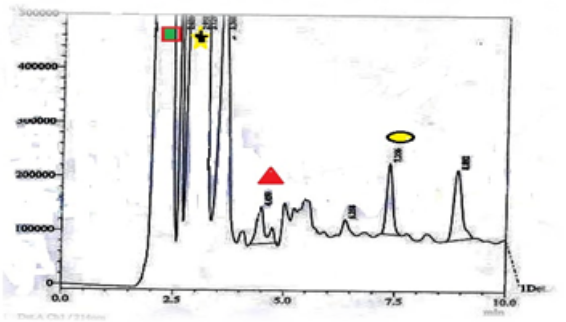

Figure (11): : HPLC curve of fruits extract

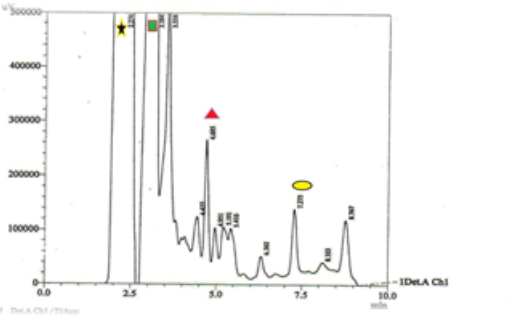

Figure (12) : HPLC curve of bark extract

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