Chapter three

RESULTS AND DISCUSSION

Preliminary screening of extracts from *"Ziziphus spina - Christi "* leaves 3-1

preliminary screening with TLC from ethanol,chloroform ethylacetate and butanol extracts , obtained from the leaves .the concentrated extracts were screened on Silica gel plates using various solvent (system (section 2-3-1-f

Examinations of the developed plates were carried out by detection under long and short wave light and spraying with spraying reagents . (plate No. (3-1))

3-1-1Screening of Ethanol Extract :

the screening of ethanol extract of leaves was studied using solvent system No.9 and 10 with spray reagent vanillin .H_2SO_4. indicated by both revealed big spot R_f value (70) plate No.(3-1).

3-1-2 Screening of Chloroform (CHCL2) Extract :

Chromatography of chloroform **extract** was studied using , solvent system No. 9 and 10 revealed "three spots" they both showed of non – polarity chracateristics R_f values (72 , 75and83) plate No . (3-1) .

3-1-3 Screening of Ethylacatate Extract :

The screening of ethylacetate extract was studied using solvent system No.9 and 10 indicated that revealed considerable number of spots " eight spots " their R_f values (3-1) R_f,(94,88,76,70,59,46,35,25)using variuose reagnts see table (3 – 1) and plates No.(3-1), No.(3-2) .

3-1-4 **Screening of Butanol Extract:**

The chromatography of butanol extract dissolved in methanol and applied on silica gel plate

using solvent system No. 9 and 10 was carried out , the revealed showed presence of many compounds "five spots " their R_f (92,78,70,59,35) see plat No.(3-1) .

From this preliminary thin layer chromatography screening , it was clear that ethyalacetate extract have rich and revealed the presence of many secondary metabolite compounds . see table (3-1) .

According to above results the ethylacetate extract was chosen for only do detailed phytochemical investigations.

Table (3-1):
Results of sprayRaegentsonTLCofethylacetateextract

Test applied	**Solvent system**	**Colour reaction**	**R_f value *100**	**Results**
Dragen dorff's Mayer's test	Chloroform: Ethyl acetate : Methanol 6:2:2v/v	-	-	Negative
5%Methanolic Potassium hydroxide	Ethylacetate Methanol (7:3 v/v)	Yellow	73	Indicated presence of Flavonoids
3% Aluminum Chloride in Methanolic	Choloroform Ethylacetate: Metharol (6:2:2 v/v)	Dark Yellow	74	Flavonoids
Ferric Chloride	Chloroform Methanrol (8:2v/v)	Green yellow	72	The formation green yellow colour was taken an evidence for presence of Tannins or Flavonoids
Vanillin in Sulphuric acid	Chloroform: Ethylacetate: Methanol (6:2:2 v/v)	Yellow	73	Flavonoids
Ninhydrin	Chloroform Methnol	-	-	-

3.2 Identification of Compounds from Ethylacetate fraction :

Three compounds; A,B and C were obtained from the ethylacetate extract of leaves as described in materials and methods.see plate No.(3-3)

3.2.1 Identification of compound A:

Compound A"40 mg." was obtained as yellow powder which also yellow under UV.
R_f values (73,70,72,) in solvent system No .14,15 and 16, thire melting point "80°C"and yellow colour when sprayed with KOH ,$ALCI_3$ and vanillin .sulphuric acid Plates No.(3-4,3 - 5 and 3-6), which is characteristic indicating feature of Flavonoids .

Negative test was given with Dragendroff's , Mayer's and Nin hydrin reagents.

The Ultravilot spectrum in methanol showed four absorption maximum 435,355,257,and 206nm "Fig" No. (3-3) and table (3-5).

In the Infrared spectrum bandes are listed as table (3-2).

Negative result was shown with Dragendaffs, Mayer,s and Ninhydrin indicating the absence of alkaloid and other Nitrogen compounds .

By comparion of m,p TLC and data of IR ,Compound A was an indictation for Flavonoid (John , et al.1973.

Table (3-5):
IR data for compound A

Cm-1	Functional group	Strength	Comment
3400	O-H	S	H-bond (Hydrogen bond)
2910	C-H	M	Aliphatic
2350	C-H	W	Aliphatic
1600	C=C	S	
1480	C=C	S	
1400	C=C	S	
1040	C-O	S	
890	C-H bending	W	Aromatic C-H deformation

Key:
S**:** strong
M: minimum
W: weak

3-2-2 Identification of Compounds B and C:

Compounds B and C gave same results which are obtained as yellow solid , have same melting point "79C" their R_f values "73,72,71" "73,72,72" "72,71,71" respectively in solvent system No. 14,15 and 16 . they appeared in yellow color under UV_{254} and dark yellow under UV_{366} .

They gave positive test with $ALCl_3$, KOH and Vanillin H_2SO_4 reagents test as indication for Flaovonoids , (see plates) No(3-4,3-5and3-6).

Negative test with Dragendorff's and Ninhydrind ,the UV spectrum, showed four absorption maximum for compound "B" at 435,354,257 and 205nm. for compound "C" at 435,353,257 and 205 nm. Suggesting the presence of aromatic ring and ether .

In the Infrared spectrum, band are listed as table (3-3) and (3-4) .Negative results with Dragendorff's, Mayer's and Ninhydrin reagnts test indicated also in the absence of alkaloid and other Nitrogen compounds .

From above analysis it's clear that compounds "B" and "C", as the similarity of features "exhibited" that their near absorption at UV and nearly peaks of Infrared see table (3-3)and(3-4) .By compression of m.p , UV, TLC and data of IR compounds B and C they could be Identifiedas Flavonoids . (John ,et al. 1973).

Table (3 - 6):
IR data of compound B

Ucm^{-1}	Function group	Strength	Comment
3400	O – H	S	H – bond
2910	C – H	S	Aliphatic
2370	C – H	W	Aliphatic
1740	C = C	M	Aromatic
1600	C = C	S	Aromatic
1400	C =C	S	Aromatic
1050	C – O	S	
990	C - H	W	Aromatic deformation
540	$(CH_2)_n$	M	Aromatic deformation

Key:
S**:** strong
M: minimum
W: weak

Table (3 - 7):
IR data of compound C

Ucm^{-1}	Function group	Strength	Comment
3400	O – H	S	H – bond
2915	C – H	S	Aliphatic
2360	C – H	W	Aliphatic
1750	C =C	M	Aromatic
1610	C = C	S	Aromatic
1400	C = C	S	Aromatic
1050	C – O	S	
980	C – H	W	C- H aromatic de formation
550	$(CH_2)_n$	M	C- H aromatic deformation

Key:
S**:** strong
M: minimum
W: weak

Since the complete study and we of documented structure elucidation. Is not possible , these results may be considered under available conditions.

Chapter four

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تم بحمد الله

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