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Original Article

Biological Studies of Flavonoids from Flowers and Herb of *Zinnia Pauciflora* Plant L.

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ABSTRACT

Zinnia pauciflora is a member of family Asteraceae, which contains some bioactive compounds such as flavonoids particularly, flavones and flavonols. Isolation and identification of some flavonoids from herb and yellow flowers as well as effect of the herb crude extract and yellow flowers were studied on liver and kidney functions as well as blood glucose also antimicrobial effect. The main compound in extract of the yellow head flowers is apigenin7-(4-acety)-xyloside, while the main compound, kaempferol-7-glucorhamnoside, was isolated from the extract of the aerial parts (leaves and stems). As for biological studies, the most effective extract against all microorganisms (gram positive and gram negative bacteria, yeast and fungi) was ethyl acetate fraction. On male albino rats, the crude extract of flavonoids from yellow flowers decreased serum creatinine, blood glucose, also ALT, AST and ALP remained in optimum level at all tested doses (0.7g, 1.4g and 2.8g/ Kg body weight/ day/2 weeks).

Keywords: *Zinnia pauciflora*, flavonoids, Liver function, blood glucose.

INTRODUCTION

Polyphenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity [1]. Herbs are used in many domains including medicine, nutrition flavoring beverage, dyeing repellents, fragrances, cosmetics [2]. Many Asteraceae species have been recognized to have medicinal properties and beneficial impact to health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, anti-mutagenic effects and anti-carcinogenic potential [3]. Flavonoid compounds have been isolated from many species of this family [4]. Sivakuman et al [5] on *Helichrysum bracteatum* plant. Li-Rong Tao et al and Stevens et al [6,7] on *Chrysothamnus* plant. Also, [8] isolated and identified flavonoids glycoside from *Echinops echinatus* helpful towards dyspeptic disorders. Hamed et al [9] isolated and identified flavonoids from *Echinops spinosissimus* as apigenin, hispidulin, 5, 4 dihydroxy flavone and apigenin 7-O- glucoside.

Zinnia is member of family "Asteraceae", annual, perennial and sub-shrubby plant [10], includes sixteen to twenty species. This genus contains many active ingredient groups such as sequiterpene lactones i.e., zinaflorins (elemanolides). *Zinnia pauciflora, Z. peruviana and Z. flavicoma* contain elemanolides [11.12]. Elemanolides has acytotoxic activity [13, 14]. Also, Flavonoids are major compounds in flowers and herb of *Zinnia* such as anthocyanins, flavones and flavonols [15]. Jadwiszczok and Migas [16] cited that leaves and flowers of *Zinnia elegans* contained eight flavonoids. Harborne et al [17] reported that the yellow flower of *Zinnia linearis* has been shown to contain aurones sulfurein and maritimein and the related chalcone marine, a five yellow phenolic pigment was also detected. Migas et al [18] found eight flavonoids in *Zinnia elegans*, six were isolated by column and paper chromatography and identified as kaempferol 3-glucoside, kaempferol 3-xyloside-7-glucosid and luteolin 7-glucoside. Forkman and Siotz [19] detected flavanone 3-hydroxylase in flower extracts of cyanic strains of *Zinnia*, which catalyses the conversion of flavanones to dihydroxy flavonols.

Many literature surveys revealed different pharmacological and biological activities of flavonoids. Flavonoids have anti-inflammatory action [20], anti-bacterial, antifungal effect [21]. Also they possess anti-carcinogenic effects since they can interfere with initiation, development and

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progression of cancer by the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis [22]. Also, [23] found that a novel flavonoid c-glycoside, 5-hydroxy-1-methory-6-c-glycosylfavone was isolated from the aerial parts of *Sphaeranthus indicus*, family compositae. The leaves of this plant have macrofilaricida, antimicrobial and insecticidal activities. Aneta et al [24] stated that the amount of total phenolics in *Melissa afficinalis* 13.2 mg GAE/ 100g dw, *Acorus calamus* and *Taraxacum afficinale* (12.6 mg GAE/100g dw) had very high levels of phenolics. Matthes and Honermeier [25] mentioned that, the green rosette leaves of *Cynara cardunculus* are used in pharmaceuticals. Polyphenolic compounds like caffeoylquinic acids and flavonoids are the main chemical compounds in leaves. Flavonoids protect the gastrointestinal mucosafram lesions produced by various experimental ulcer models and against different necrotic agents [26]. Flavonoids are powerful antioxidants against free radicals and are described as free radical scavengers [27].

The available literature cleared that *Z. pauciflora* has bioactive compounds which gave this species its importance as medicinal plant.

This study aimed to isolation and identification of major flavonoid from yellow flowers, and herb of *Z. pauciflora* as well as, studying the antimicrobial activity of flavonoid fractions from flowers and herb, with subchronic toxicity study of flavonoid fractions from flowers on rats.

MATERIAL AND METHODS

Materials: Paper chromatography (PC) whatman 3MM and filter paper sheets were from Whatman international Ltd. TLC plates were carried on microcrystalline cellulose LR (s.d. fine - chem. Ltd.), and used for thin layer chromatography (MCC). All solvents were technical grade (Aldrich).

Phytochemical Study

Isolation and purification of flavone from yellow flowers: According to [28].

Preparation of ethanol extract from the yellow flowers: Flowers of *Z. pauciflora* (270g) were extracted at room temperature by blending with one liter of ethanol (80%). After filtration, the residue was reblended with ethanol. The filtrates were combined and concentrated to 100m1 under reduced pressure. The solution was filtered through celite to remove cellular material. The filtrate was successively washed with benzene, light petroleum ether, and finally exhaustively extracted with ethyl acetate.

Chromatographic fractionation of the ethyl acetate extract: The ethyl acetate extract was banded on PC 3MM and fractionated by n.butanol acetic acid: water (4: 1: 1). Five distinct bands were detected under UV, and were cut off separately, then eluted with 70% ethanol. Each elute was concentrated and rechromatographed with solvent system n.butanol: acetic acid 27% (4:1).

Isolation and purification of herb flavonol.; according to [29]: Air dried powdered herb (one kg) was extracted by percolation with ethanol 80%. The extract was successively washed with hexane, petroleum ether (40-60°C), ether and then chloroform. Fraction was evaporated under reduced pressure to small volume (20ml). The ethyl acetate fraction was spotted on PC 3MM and separated by two dimensional paper chromatographic technique with n. butanol: acetic acid: water (4: 1: 5) and acetic acid 15%. The spots were detected using UV lamp after exposure to ammonia vapor. One main spot was eluted then purified by ascending paper chromatographic technique. In order to obtain more information about chemical constituent of the isolated compound, the following analyses were carried out, color under UV, shift with $ALCl_3$, HCl, NaOAc, H_3BO_4, NaOMe and the acid hydrolysis was carried out to investigate the sugar moiety. The ^{1}H-NMR analysis was by nuclear magnetic resonance (Varian Gemini 200MHz) and the mass spectrometer (Finningen SSQ 7000) was also applied in the following.

Biological studies

Antimicrobial effects of flavonoids from flowers and herb of Zinnia pauciflora as crude extracts

Microbial material: Pathogenic fungi were *Fusarium oxysporium* (NRC 1) and *Aspergillus niger* (NRC 2) while the yeast was *Candida albicans* (NRC 3). The tested bacteria were classified into two groups; Gm^+ bacteria, *Bacillus subtilis,* and Gm^-bacteria, *Escherichia coli.* Yeast, fungi and bacteria were supplied by Natural and Microbial Products Laboratory, National Research Centre.

Media: The following media were used; Potato-Dextrose Agar growth medium (PDA) for culturing the fungi, according to [30], Lauria- Bertani medium (LB medium) for culturing the bacteria according to [31] and Yeast Extract Peptone Medium (YEPD) for culturing the yeast according to [32].

Technique for antimicrobial test: The antimicrobial assay was disc diffusion method against fungi, yeast and bacteria was according to [33].

Biological studies on rats

Experimental animals: The residue after ethyl acetate evaporation (0.7g, 3.6g and 7.2g) was treated separately with 20m1 tween (80) to give an emulsion. Each rat was daily ingested by stomach tube with 1ml tween-extract emulsion for 4 weeks. Male albino rats (20) from animal house of National Research Centre weighing 100- 150g were used in this experimental. The animals were classified into four groups, each group contained six rats.

1. The first group was used as –ve control (ingested saline daily)
2. The second group was daily ingested with 0.3m1 of tween solution equivalent to (70 mg extract /kg B.wt)
3. The third group was ingested with 0.5m1 of tween solution equivalent to (140 m extract/kg B.wt.
4. The fourth group was ingested with 0.5m1 equivalent 280 mg/kg B.wt.

Blood samples were collected from eye blood vein. Sera were separated and the following investigations were carried out:

- Liver function tests in serum including asparatate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as well as Blood glucose
- Kidney function tests included blood urea and serum creatinine.
- Kits for biochemical assay were from Bio Merieux, France.
- LD_{50} was calculated according to method of [34].

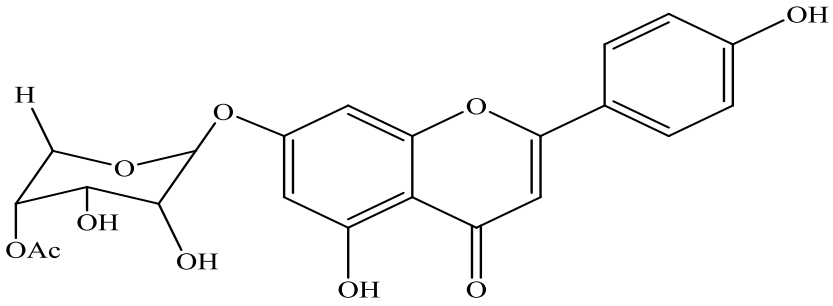
RESULTS AND DISCUSSION

Identification of Flavone isolated from yellow head flowers of Zinnia pauciflora: Compound (A) was isolated from yellow flowers of *Zinnia pauciflora* as shown in material, and detected with different solvent systems, where it gives single spot. The R_f values were 0.25, 0.23 and 0.65 with 15% acetic acid, 50% acetic acid and n. butanol: acetic acid: water (4: 1: 1), respectively.

UV spectrum: The UV spectral data showed two absorption bands, band I at 300- 380nm and band II at 240- 280nm in methanol. These absorption bands are identical to the flavone apigenin. Band II (273nm) and shift at band I in sodium methoxide (40nm) indicate C-4'-OH group. The shift of band II in sodium acetate (20nm) denoted the presence of free hydroxyl group at C4' and non-free hydroxyl group at C-7. The isolated compound A did not show characteristic shift in aluminium chloride at band I, this indicates the absence of ortho- hydroxyl group with respect to the present free hydroxyl group at C-5, Table (1).

Table (1): The UV spectral properties of compound A from yellow flowers

	Spectral maximum					
Compound A	**MeOH**	**NaOMe After l0min**	**Al $C1_3$**	**Al $C1_3$+HC1**	**NaOAc**	**NaOAc + H_3BO_4**
	273, 332	274, 305, 371	276, 308, 352	276, 300, 342, 380	273, 351	273, 326, 351



Apigenin 7-(4``-acetyl)-xyloside

^{1}H-NMR spectrum: The high field region showed the following signals; signal at 1.904ppm for acetyl group at xylose moiety and no signal at 2.50ppm indicates the absence of acetate of aromatic nucleus as reported by [29] for acetyl radical on sugar nucleus, signal at 3.31ppm for H-2", 3.32ppm for H-5", 4.64ppm for H-1 as well as 4.85ppm and 4.905ppm for protons of sugar hydroxyl groups and signal at 8.55ppm for H-2'.

Mass spectrum: The mass spectral data of compound A showed main fragment at m/z 60 as base peak due to removal of acetic acid in an ionic fragment, then removal of one hydrogen proton showed peak at m/z 395. In other way of fragmentation, peak at m/z 412 indicates the removal of the sugar moiety, peaks at m/z 147 and m/z 183 for attachment of the aglycone with pentose sugar (xylose). This fragmentation path way is according to **[35]**.

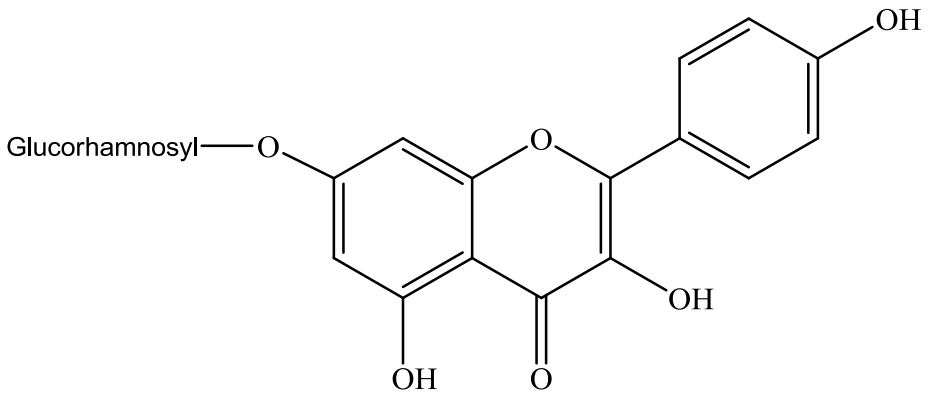
IR spectrum: The infrared spectrum revealed band of -OH at 3417.24cm^{-1}, 2840 cm^{-1} and 2960 cm^{-1} of -CH and -CH_2, 1680 cm^{-1} for =CO group, band at 1400 cm $^{-1}$ for-CH_3 of acetate and 1016 cm $^{-1}$ for acetyl group. The data explained above showed the position of acetyl radical on the sugar moiety as reported by [35] and the presence of peaks in the mass spectrum at m/z 412 and 395 suggested most probably the presence of one xylose molecule with one acetyl group. However the specific position of the acetyl group on any OH of the sugar is out of our facilities in the present work and needs more investigation in a further study.

Identification of flavonol isolated from herb of Zinnia pauciflora: Compound B has a yellow color under UV light. It is completely soluble in methanol and ethyl acetate. It gives single spot with different solvent systems. The spectral data (UV and ^{1}H-NMR) of this compound indicated that, it is related to kaempferol glycoside. The acid hydrolysis of this compound revealed its sugar moiety to be glucose and rhamnose, this was supported by the ^{1}H-NMR spectral data. ^{1}H-NMR spectrum (CD_3OD) showed as follows, signals at 1-1.29ppm (rhamnose-CH_3), 3.30- 3.32ppm (H-2, H-5, H-6 of glucose and H-5 of rhamnose), 4.79ppm (H-1 glucose), while signal at 4.637ppm for H-1 rhamnose. Protons of sugar hydroxyl group give signals at 4.80- 4.94ppm. The ^{1}H-NMR of aglycone in showed main signal at 5.64ppm (H-6 proton), 7.39ppm for H-5', 7.43ppm of H-3', 8.20ppm for H-6' and signal at 8.57ppm for H-2'.

UV spectrum: The UV spectra indicate that the aglycone is kaempferol, it gives band I at 299nm and band II at 260nm. The bathochromic shift from 310 to 360nm in band II with sodium methoxide indicate the presence of free -OH at C-4' and free OH group at C-3. The none free -OH at C-7 (attached with sugar moiety) showed no change with sodium acetate and aluminium chloride reagents. This systematic identification is in accordance with [29].

Table (2): The UV spectral properties of compound B from herb of *Zinnia pauciflora*

Compound B	Maximum absorption					
	MeOH	**NaOMe After 10 min**	**Al $C1_3$**	**Al $C1_3$+HC1**	**NaOAc**	**NaOAc + H_3BO_4**
	262, 299, 310	262, 299, 360	262,299,366	262,299,324,359, 363	262,299,310	262, 280, 319,330



Kaempferol-7-glucorhamnoside

Effect of yellow flowers extracted from Zinnia pauciflora on rats: The yellow flowers were extracted with ethyl acetate and the residue was used in treating animals at different doses (0.7, 1.4 and 2.8g/kg b.wt.) for two and four weeks to observe any deleterious effect on experimental animals. Data shown in Table (3) show that low dose of *Zinnia* extract did not give any deleterious effect whereas dose of 1.4mg/ kg body weight decreased serum creatinine and blood glucose. The administration of high doses (1.4, 2.8g/kg b.wt.) for 4 weeks caused increments in asparatate transaminase, alanine transaminase and alkaline phosphatase activities (P<0.05). These doses significantly reduced serum creatinine, blood urea and blood glucose. These results are in accordance with those of [36] who reported that, administration of high dose (83.1 mg/100 g b.wt.) of flowers ethanol extract led to a significant elevation of alkaline phosphatase activity, AST and ALT. The same dose significantly reduced creatinine. The findings of increased levels of AST and ALT are indicative of hepatic dysfunction and my due to necrosis of the liver cells. These changes may be due to coumarins of *Zinnia elegans.*

Table (3): Blood glucose, kidney function and liver function of rats treated with crude extract of yellow flowers for 4 weeks.

Dose	Two weeks						Four weeks					
	Blood Glucose mg/dl	Blood Urea mg/dl	Serum creatinine mg/ dl	ALT IU /ml	AST IU /m1	ALP IU /m1	Blood Glucose mg/dl	Blood Urea mg/dl	Serum creatinine mg/ dl	ALT IU /ml	AST IU /m1	ALP IU /ml
Control	97.00	33.00	0.58	44.00	50.00	22.00	96.00	34.00	0.59	43.00	50.00	23.00
0.7 g/kg B.wt	67.50	33.00	0.57	45.00	50.50	21.30	95.00	33.00	0.57	44.00	51.00	22.00
1.4 g/kg B.wt	96.80	32.00	0.58	45.50	52.00	22.28	93.00	32.00	0.54	46.00	53.00	27.00
2.8 g/kg B.wt	95.00	32.00	0.65	47.00	53.00	24.49	91.00	32.50	0.49	48.50	58.00	34.00
L.S.D. (5%)	1.90	1.01	0.02	1.42	1.51	0.82	2.10	1.30	0.02.	1.44	1.73	0.92

Data are presented as mean of triplicates.

Effect of crude extracts* of *Zinnia pauciflora against different microorganisms: The crude extract of herb flavonoids and yellow flowers of *Zinnia pauciflora* were tested for their antimicrobial effect at three concentrations, 100**,** 200 and 300ppm using disc papers (6mm) which were impregnated with the extracts. Data in Table (4) indicate that the two extracts showed inhibition activity against gram negative and gram positive bacteria. The crude extract of flower was more effective than crude extract of herb against *B. subtilis* at all tested concentrations 100ppm, 200ppm and 300ppm. Flowers crude extract appeared to be more effective against *E. coli* at all concentrations. The crude extract of flower was more effective against gram negative and gram positive bacteria than herb extract at all concentrations. The growth of yeast (*Candida albicans)* was inhibited by herb crude extract of herb and flowers at all concentrations, but extract of flowers was potent against yeast than extract of herb at all concentrations (100, 200, 300ppm).

Data in Table (4) indicate that crude flowers extract was more effective against *A. niger* and *F. oxysporium* than herb extract at all concentrations (100, 200 and 300ppm). The significant inhibitory effect of crude extracts of flavonoids from flowers and herb on the growth of microorganisms may be attributed to the presence of apigenin -7- xyloside and kaempferol -7- gluco rhamnoside, which were isolated from this extracts. These results are in accordance with those of [37], who stated that ethyl acetate extract of *Heliotropium digynum* showed a great acivity against *B. anthrocoid* and moderate activity against *Candida albicans, E. coli* and *Salmonella typhi.* Apigenin4'-glucosyl 7glucoside, kaempferol 3- glucoside, kaempferol 7- glucoside and kaempferol 3, 7 di glucoside were isolate from this exract. The effectivity of our extracts showed results which agreed with those of [38]**,** who reported that the ethyl acetate extract of *Hyaloxylon schmittiana* contained kaempferol. This extract showed great activity against *B. subtilis, E. coli, A. niger* and *Candida albicans.* Our results are also in accordance with [39] who cited that apigenin inhibited gram positive bacteria *(Staphylococcus aureus)* and apigenin 7- triglucoside inhibited gram negative bacteria *(E. coli)* at minimum inhibitory concentration.

Table (4): Antimicrobial activity of crude extractes from herb and flowers of *Zinnia pauciflora*

Kind of M.O.	Diameter of inhibition Zone in mm							
	Crude extract of herb			L.S.D.	Crude extract of yellow flowers			L.S.D. (5%)
	100 ppm	200 ppm	300 ppm	(5%)	100 ppm	200 ppm	300 ppm	
1- *B. subtilis*	7.00	11.25	19.00	3.64	8.00	17.00	19.25	2.14
2- *E. coli*	6.00	7.00	8.00	0.61	9.00	10.75	20.75	1.21
3- *Candida*	7.30	10.00	11.00	1.53	7.60	10.00	14.30	1.53
4- *A. niger*	9.30	11.16	13.80	1.24	10.33	11.83	16.00	1.65
5- *F. oxysporium*	8.00	11.16	14.16	1.60	10.00	12.50	15.50	1.62

Data are presented as mean of triplicates

Conclusion: The main compound in extract of *Zinnia pauciflora* yellow head flowers is apigenin7-(4-acety)-xyloside, while the main compound in extract of the aerial parts which include leaves and stems was kaempferol-7-glucorhamnoside. As for biological studies, the most effective extract against all microorganisms was flower extract (garm positive and gram negative bacteria, yeast and fungi). On male albino rates, the crude extract of flavonoids from yellow flowers decreased serum creatinine, blood glucose, ALT, AST and ALP at 0.7g and 1.4g /kg b wt through 4 weeks, while the high dose 2.8g/kg b wt/4 weeks increased AST and ALT proved a liver dysfunction.

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