EFFECT OF POLYPHENOLIC COMPOUNDS ISOLATED FROM *NANDINA DOMESTICA*. THUNB. LEAVES GROWING IN EGYPT AGAINST INDUCED ECZEMA IN MICE.

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Abstract

Herbal therapy is considered as the new world attitude; this might be attributed to the safety offered by the natural medicinal products with respect to the traditional synthetic therapeutic agents. Berberidaceae, family comprising 14 genera and 701 species of perennial herbs and shrubs. Phytochemical investigation of Ethyl acetate fraction of dried leaves of *Nandina domestica* led to the isolation of four compounds (1 -4). The isolated compounds were identified by their NMR, MS spectral data analysis as caffeic acid (1), chlrogenic acid (2), quercetin (3) and rutin (4). This is the first report of isolation of chemical entities from leaves of this plant. Ethyl acetate fraction of *Nandina domestica*, exhibited antieczyma activity against induced eczema in mice

Keywords: *Nandina domestica*, antieczyma, caffiac acid, chlrogenic acid, quercetin rutin.

Introduction

One of important members of interest include *Nandina domestica* Thunb. The only species in the genus. *Nandina domestica* Thunb. It is a monotypic genus(Bi.*et al*,2015). Phytochemical analysis of various species of genus revealed the presence of alkaloids, tannins, phenolic compounds, sterols and triterpenes. (Mokhber: ;2013). Eczema (Dermatitis) is mostly acute, less frequently chronic, recurrent, non-contagious inflammatory condition of the skin; it represents an allergic response to a variety of agents that react on the skin from either the outside or the inside. It is characterized by redness, itching, blistering, oozing, weeping; scaling, thickening of skin. (Skripkin Y.K. *et al.,* Abdallah M.A.-R., *et al.,* (1994), Phelps R.G., *et al.,* (2003) &Mohammed A. Mahdy, (2004). Acute eczema is presented as ill-defined erythematous edematous patches that may show papules, vesicles, oozing areas, or crusts. Due to the diversity of causes of eczema and types of reaction, treatment should be planned to suit each case individually. It is important to try to find the possible cause and to tell the patient how to avoid it. The following are the measures that may be applied in a case of eczema, Systemic therapy, Topical therapy (Feingold S., *et al.,* (1998)., Cabral P.S., *et al*., (2003). available online at http://www.hkmj.org.hk/skin/stdframe.htm), Psoralens plus ultraviolet light (PUVA), Immunosuppressive (Dattner A.M.; (2003). Hehmann M., *et al*., (2004) , Carvalho J.C.T., *et al.,* (1999).Khandpur S., *et al*., (2004), Donfack J. *et al*., (2011) Lv YJ, *et al*.,2008& Saxena N., *et al*., (1988).& Phototherapy (*WHO*

Monographs On Selected Medicinal Plants (1999),Congora L., *et al*.,(2002)&Kimata M., *et al*., (2000). Mills' S. et al., (2002) & Graf J. (2002).

Material and methods:

phytochemical study:

Leaves and of *Nandina domestica* Thunb. were collected from El-Orman botanical garden, Cairo, Egypt in 2015. The dry ground leaves of *Nandina domestica*. (1 Kg) were extracted with 70% alcohol several times, isolation and identification of the active constituents The English Text of The Egyptian Pharmacopoeia (1984) & Stahl E. (1969).

The total extract was concentrated under reduced pressure and subjected to successive fractionation with methylene chloride, ethyl acetate & butanol. ethyl acetate fraction was applied on silica gel column and eluted with CH_2Cl_2: MeOH from 95:5 to 15:85 to obtain 10 collective fractions. Fraction 5 was subjected to successive column chromatography on Sephadex LH-20 using methanol to offered four compounds **1, 2, 3, &4** which subjected to ESI –mass and ^{1}H- and ^{13}C-NMR measurements (Ain shams University), spectrometer operating at 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C).

biological studies Treatment of induced eczema in mice:

A) Preparation of the extracts and ointments: ethyl acetate fraction was used in preparation of 2%, ointments in Vaseline base.

B) Animals: Male albino mice weighing (25-30 g) were used; the animals were kept in groups of 8 animals in each polyethylene cage at room temperature. They were fed on standard chow, and allowed free access to drinking water. They were acclimatized at least for one week prior to use.

C) Preparation of dinitrochlorobenzene (DNCB): The first sensitizing dose was 2% w/v DNCB in acetone and the second one was 0.2% w/v for further application if eczema still not formed.

D) Induction of eczema: Each mice group (8) was sensitized by local application of 2% DNCB. In about 90% of the animal the eczema was formed after 4 days. To the rest of animals 0.2% w/v of DNCB solution is applied locally and the eczema was formed after 2 days.

E) Treatment of the induced eczema in mice: Groups of mice (8 each) were treated once daily with ointments of different extracts (2 % w/w ethyl acetate fraction in Vaseline) and compared with positive control (mometasone furoate ointment Elocon® ointment).

F) The data expressed as percentage of number of healed animals in test period in respect to total number of treated animals

Results and discussion

Phytochemical investigation of Ethyl acetate fraction of dried leave of *Nandina domestica* led to the isolation of four compounds (1 -4). The isolated compounds were identified by their NMR, MS spectral data analysis as caffiac acid (1), chlrogenic acid (2), quercetin (3) & rutin (4).

Compound 1

It was isolated as creamy amorphous powder. ESI-MS of the compound showed $[M-H]^+$ peak at m/z 179.1, which is compatible with the molecular formula $C_9H_8O_4$. The ^{1}H NMR spectrum of the compound exhibited characteristic signals for δ 7.57 (1H, d, *J* = 16 Hz, H-7), 7.06(1H, d, *J* = 2.0 Hz, H- 2), 6.96(1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.94(1H, d, *J* = 8.0 Hz, H-5), 6.26 (1H, d, *J* = 16 Hz, H-8), The ^{13}C-NMR spectrum of the compound exhibited signals at δ 169.80(C-9), 148.03 (C-4), 145.76 (C-3), 145.5C-7), 126.43 (C-1), 121.57 (C- 6), 115.15 (C-5), 114.11(C-2), 113.77 (C-8) From the above mentioned data, the compound identified as **caffeic acid,** which confirmed by direct comparison with published data (Kragujevac. (2017).

Compound 2

It was isolated as white amorphous powder. ESI-MS of the compound showed $[M-H]^+$ peak at m/z 353.4, which is compatible with the molecular formula $C_{16}H_{18}O_9$.

The ^{1}H NMR spectrum of the compound exhibited characteristic signals for δ 7.60 (1H, d, *J* = 16 Hz, H-7'), 7.07(1H, d, *J* = 2.0 Hz, H- 2'), 6.98(1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.81(1H, d, *J* = 8.0 Hz, H-5'), 6.30 (1H, d, *J* = 16 Hz, H-8'), 5.37(1H, ddd, *J* = 6.8, 6.8, 4.4 Hz, H-5), 4.21 (1H, ddd, *J* = 7.2, 4.19, 4.18 Hz, H-3), 3.77(1H, dd, *J* = 6.8, 3.75 Hz, H-4), 2.28 (1H, m, H-2ax), 2.23 (1H, m, H-6ax), 2.12 (1H, dd, *J* = 13.2, 4.0 Hz, H-6eq), 2.10 (1H, dd, *J* = 13.2, 7.6 Hz, H- 2eq)

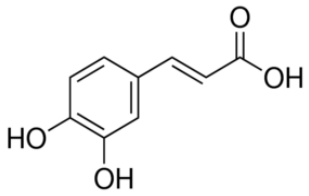
The ^{13}C-NMR spectrum of the compound exhibited signals at δ 175.64(C-7), 167.31 (C-9'), 148.16 (C-4'), 145.72 (C-3'), 145.38 (C-7'), 126.41 (C-1'), 121.63 (C- 6'), 115.11 (C-5'), 113.87 (C-2'), 113.84 (C-8'), 74.77 (C-1), 72.10 (C-5), 70.57 (C- 4), 69.92 (C-3), 37.40 (C-2), 36.81 (C-6). From the above mentioned data, the compound identified as **chlorogenic acid**, which confirmed by direct comparison with published data (Jelena T. *et al.*, 2017)

Compound 3

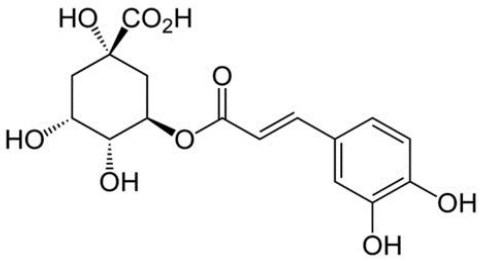
The compound was obtained as a yellow amorphous powder. The ESI-MS of the compound showed a peak at m/z 302.0 for $[M+H]^+$ indicating that the molecular formula is $C_{15}H_{10}O$. The ^{1}H-NMR spectrum of the compound showed a clear flavonol type pattern of aromatic proton signals; two meta coupled protons each at δ 6.19 ppm (d, *J*= 2.0, H-6) and δ 6.41 ppm (d, *J*= 2.0, H-8), doublet of one proton at δ 6.90 ppm (d, *J*=8.4 Hz, H-5$^{)}$) and a meta coupled doublet of one proton at δ 7.68 ppm (d, *J*= 2.0, H-2$^{`}$) and H-6' appear as doublet of doublet at 7.55 ppm (dd, *J*= 8.4, 2.0, H-6$^{`}$). This data indicated that the compound is a 3, 5, 7, 3, 4$^{`}$ –penta oxygenated flavone derivative, which is in a good agreement with Quercetin (Markham, K. R 1982, Mabry, T. J. *et al*., 1970). The ^{13}C-NMR spectrum of the compound supporting the structure assignment made above, revealing the presence of 15 carbon signals, including 8 oxygenated at [δ 145.11 (C-2), 160.78 (C-5), 163.97 (C-7), 156.21 (C-9), 146.86 (C-3$^{`}$), 135.78 (C-3), δ 175.89 (C-4) and 147.75 (C-4$^{`}$)]. 7 non-oxygenated at [δ 98.26 (C-6), 93.42 (C-8), 103.07 (C-10), 122.04 (C-1$^{`}$), 115.14 (C-2$^{`}$), 115.67 (C-5$^{`}$) and 120.05 (C-6$^{`}$)] (Harborne, J. B. 1994, Markham, K. R. et al., 1978& Agrawal, P. K.: 1989). On the basis of the above mentioned data and by comparison with that reported in literature, the compound **was** identified as **Quercetin**.

Compound 4

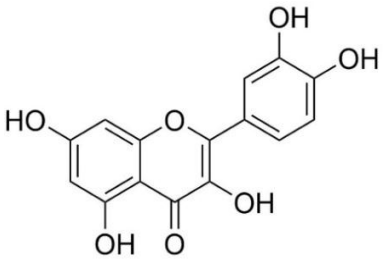
This compound was obtained as a yellow powder. The negative mode ESI-MS of compound showed a peak at m/z 609.2 for $(M-H)^-$, this signal correspond to the molecular formula $C_{27}H_{30}O_{16}$. The ^{1}H-NMR spectrum of the compound showed two meta-coupled doublets of one proton each at δ 6.19 ppm (d, *J* = 2.0 Hz H-6) and δ 6.39 ppm (d, *J* = 2.0 Hz H-8), one ortho-coupled doublet of one proton at δ 6.85 ppm (d, *J* = 8.8 Hz, H-5`), a douplet of douplet of one proton at δ 7.53 ppm (*dd*, *J* = 7.2, 2 Hz H-6`) and a meta-coupled douplet of one proton at δ 7.55 ppm (d, *J* = 2.4 Hz H-2`). These data indicated that the compound was a 3,5,7,3`,4`-penta-oxygenated flavonoid derivative, which is in good a greement with quercetin. Its ^{1}H-NMR spectrum exhibited two anomeric proton signals at δ 5.35 ppm (1H, *d*, *J* = 7.2 Hz, H-1`` of glucose) and δ 4.39 ppm (1H, *d*, *J* = 1.6 Hz, H-1``` of rhamnose) which were consistent with the configurations β for D-glucose and α for L-rhamnose, respectively. In addition, the apppearance of the strong sharp douplet at δ 1.002 ppm (3H, *d*, *J* = 6.4 Hz, secondary-CH_3 of rhamnose) confirmed the rhamnose unit. The chemical shift value of the anomeric proton of glucose (δ 5.35 ppm) supported that the glucose attached to aglycone on the other hand, the chemical shift value of the anomeric proton of rhamnose (δ 4.39 ppm) confirmed that the rhamnose unit must be linked to the glucose moiety (Markham, K. R 1982, Mabry, T. J. *et al*., 1970).The ^{13}C-NMR spectrum also supported the structure assignments made above and, in particular, confirmed the presence of one rhamnose moiety followed from the signal of a methyl group (δ 17.75). The biglycosidic sugar moiety must be attached to the position C-3 of the aglycone, due to the upfield shift (δ 133.33) of this carbon signal in comparison to that of quercetin (C-3, δ 135.6; *O*-glycosylation effect).The attachement of the rhamnose to C-6`` of glucose moiety was also deduced by $^{13}$C-NMR spectrum due to the downfield shift of the glucose C-6`` signal from 61.0 to 67.02 ppm (due to rhamnosylation at C-6``), (Harborne, J. B. 1994, Markham, K. R. et al., 1978& Agrawal, P. K.: 1989). . On the basis of the above mentioned data, this compound was identified as **Quercetin-3-*O*-Rutinoside (Rutin).**



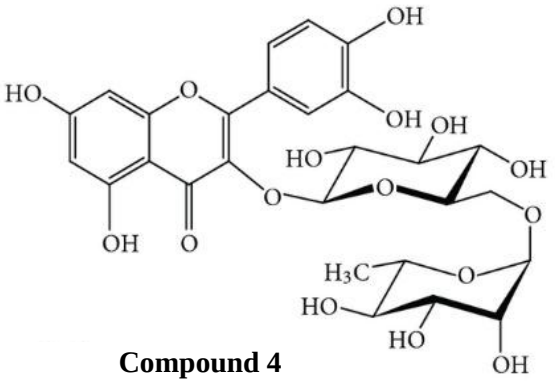
Compound 1



Compound 2



Compound 3



Compound 4

Anti-eczematic activity results:

A placebo-control trial comparing the ointments were prepared from the ethyl acetate fraction, ***Nandina domestica*** with 0.1 % mometasone furoate ointment and placebo of vaseline. Treatment was continued until complete recovery of the induced eczema. The animals were investigated every day and the cured animals were recorded. At the end of the test period the total number of the cured animals was recorded. Results are listed in table below.

Anti-eczematic activity of 2% w/w ethyl acetate extract of *Nandina domestica* against 0.1% w/w mometasone furoate and vaseline (placebo).

Days of treatment	No. of cured mice		
	2% ethyl acetate of *Nandina domestica*	0.1%mometasone furoate (standard)	Placebo (Vaseline)
1st	0	0	0
2nd	0	0	0
3rd	0	0	0
4th	1	2	0
5th	2	1	0
6th	2	1	0
7th	1	2	0
8th	1	0	0
9th	0	0	0
10th	0	0	0
Total/group N = 8	7	6	0
% of cure	87.5%	75%	0%

Conclusion:

Study of the effectiveness of ***Nandina domestica*** leaves ethyl acetate extract in management of the induced eczema revealed that, ***Nandina domestica*** leaves ethyl acetate extract was more effective than placebo and 0.1% mometasone furoate in treatment of induced eczema in mice.

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الفاعلية المضادة للأكزيما المستحثة فى الفئران

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يتجه العالم حديثا للعلاج بالأعشاب لأنه أكثر أمان من الأدويه المصنعه، نبات ناندينا دوماستيكا الذي ينتمي لعائله باربرداسي تم استخدامه في الطب التقليدي كثيرا، في هذه الورقه البحثيه تم عمل المسح الكيميائي علي خلاصة خلات الاثيل لأوراق شجرة ناندينا دوماستيكا حيث تم فصل أربع مركبات وهم (حمض الكافيك، حمض الكلوروجينيك، الكوارسيتين و الروتين).

كما تم أختبار الفاعلية المضادة للأكزيما المستحثة فى الفئران (مايس) لأجزاء خلات الايثيل وذلك بتحضير مراهم بتركيز ٢% وباستخدام فيورات الموميتازون كمحكم إيجابي والفازلين كمحكم سلبي وقد أثبتت التجربة أن تركيز ٢٪ مرهم خلات الايثيل فعال في علاج الأكزيما بالمقارنه مع مرهم فيورات الموميتازون.