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Phenolic Investigation and Potential Therapeutic Activities of *Alectryon tomentosus* (F.Muell.) Radlk Leaves Family Sapindaceae

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Abstract:

The first phytochemical investigation of *Alectryon tomentosus* (F.Muell.) Radlk leaves led to the isolation and identification of nine compounds. Two phenolic acids; 4-hydroxy benzoic acid (1) and gallic acid (2) and one flavonoid aglycone; luteolin (3) were isolated from the methylene chloride extract. Also another flavonoid aglycone; myricetin (4) was isolated from the ethyl acetate extract. Moreover, four flavonoids glycosides; luteolin-7-*O*-*β*-glucoside (5), luteolin-7-*O*-*β*-rutinoside (6), myricetin-3-*O*-*β*-glucoside (7) and myricetin-3-*O*-*α*-rhamnoside (8) and one flavonoid aglycone; velutin (5,4'-dihydro-3',7-dimethoxy flavone) (9) were isolated from the butanol extract. Their structures were established on the basis of chromatographic and spectroscopic analysis which is consistence with previous published literature. The first biological investigation of *A. tomentosus* leaves extracts highlighted their potential therapeutic benefits. The extracts were evaluated for their antioxidant activity by determination of blood glutathione level, anti-inflammatory activity using carrageenan induced paw edema model, analgesic activity using both hot plate technique and writhing test and antiulcer activity using Corell test in albino rats. Methylene chloride, ethyl acetate and butanol extracts exhibited significant antioxidant activities. Both methylene chloride and aqueous extracts exhibited significant anti-inflammatory activities, whereas, methylene chloride, ethyl acetate and aqueous extracts exhibited significant analgesic and antiulcer activities. This study is valuable for understanding the potential therapeutic importance of natural flavonoids and phenolics. It is worth to mention that there are no previous phytochemical or bioactivity studies reported for *A. tomentosus*.

1. Introduction

Phenolic compounds, in natural matrices, are object of profound interest, in what concerns their chemistry and their interesting biological and pharmacological properties [1]. Previous studies proved that phenolics are able to act as antioxidants in a number of ways [2]. Several phenolic and flavonoid compounds from plant extracts have been found to possess antioxidant, antimicrobial, anti inflammatory, analgesic and antiulcerative properties in various studies [3-8]. Despite the reported medicinal benefits of phenolics, there is a slow progress of new drug registration observed in that category of natural products [9]. The present study, aims at the exploration of the potential therapeutic uses of a plant enriched with these compounds.

A. tomentosus (=*Nephelium tomentosum* F. Muell.) is a species of trees in the soapberry family; Sapindaceae that occurs in rainforests. One common name, hairy bird's eye, refers to the shiny black seed exposed in the fleshy red aril [10, 11]. To our knowledge, *A. tomentosus* has not been the subject of any study.

In the present study, we isolated and identified the major phenolic constituents that are believed to be correlated with the present biological activities. The study included the following biological activities; antioxidant activity was visualized by its scavenger ability, anti-inflammatory activity by examining the reduction of the carrageenan-induced paw swelling, whereas, analgesic activity is evaluated by writhing and hot plate test. In addition, the gastroprotective activity is established by comparing the number of ulcers or erosions to control.

2. Experimental

2.1. Plant material

Fresh leaves of *A. tomentosus* were obtained from Orman garden, Giza, Egypt, in April 2014. The plant was identified by Mrs. Therese Labib, plant taxonomist at Orman Garden, Giza, Egypt and confirmed by the taxonomist, Dr. M. El-Gebaly, NRC. The voucher specimen number M129 was deposit by Dr. Mona Mohamed Marzouk in the herbarium of National Research Center (CAIRC). The leaves of the plant were air-dried, powdered and kept in tightly closed containers.

2.2. Chemicals

Petroleum ether 60-80°C, ethanol and acetic acid were purchased from Adwic (Egypt). Methylene chloride, ethyl acetate and butanol were obtained from Sigma Aldrich (USA). Luteolin, myrectin, myrectin-3-*O*-rhamnoside, myrectin -3-*O*-glucoside, rhamentin, vitexin, quercetin and gallic acid were obtained from the department of phytochemistry and plant systematics, NRC. Shift reagents and chemicals for UV spectroscopic analysis of flavonoids were prepared according to Markham [12]. Sephadex LH-20 (25-100 µm) was purchased from Sigma-Aldrich Chemie GmbH (Germany). Indomethacin (EPICO., Egypt) was used as standard anti-inflammatory, analgesic drug and for ulcer induction. Carrageenan (Sigma Co.) was used for the induction of acute inflammation in rats. Acetic acid: was used for the induction of pain in rats Alloxan (Sigma Co) was used for the induction of diabetes in rats. Vitamin E (dl α-tocopheryl acetate) (Pharco Pharmaceutical Co, Egypt), gelatinous capsules, each contains 400 mg vitamin E, as a reference antioxidant drug.

2.3. Instruments

Spectrophotometer used for detecting λ_{max}in MeOH and shift reagents (UV-VIS double beam UVD-3500 spectrophotometer, Labomed, Inc.). NMR experiments were recorded on Jeol EX-500 spectroscopy: 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR)-and Varian Mercury VX-300: 300 MHz (^{1}H-NMR).

2.4. Extraction

One and half Kilogram of *A. tomentosus* air dried leaves powder was extracted with 80% MeOH yielding 135g extract. This extract was successively extracted with petroleum ether, methylene chloride, ethyl acetate, butanol and distilled water to yield 7 g, 30 g, 13 g, 23 g and 10 g, respectively.

2.5. Isolation and purification of chemical constituents

After successive fractionation of 80% MeOH leaf dry extract with petroleum ether, methylene chloride, ethyl acetate, butanol and distilled water. Methylene chloride fraction was fractioned on silica gel column using gradient eluting system n-hexane: ethyl acetate. Moreover, ethyl acetate fraction was fractionated on silica gel column using gradient eluting system methylene chloride: methanol. Butanol fraction was fractionated on polyamide column using gradient eluting system H_2O: methanol. Eventually, upon spotting on PC Whatmann no. 1 in solvent systems BAW, 15% acetic acid and phenol, the promising fractions was selected for isolation and purification of compounds on Sephadex column (MeOH:H_2O in ratio 9:1, 8:2, 7:3 and 6:4). The selected fractions for methylene chloride were n-hexane:ethyl acetate (8:2, 1:1 and 1:9), while those selected from ethyl acetate fraction were methylene chloride: methanol (95:5 and 9:1). Furthermore, H_2O: Methanol (9:1, 3:1, 7:3, 2:1 and 6:4) were selected from butanol fraction. Similar sub-fractions were pooled together and further purified on Sephadex: fraction (10:1) using 30% MeOH to yield 2 phenolic acids, 3 flavonoid aglycones and 4 flavonoid glycosides.

2.6. Animals

Adult albino rats, of Sprague Dawley Strain weighing 130-150 g and Albino mice weighing 25-30 g. Animals were obtained from the animal house colony of the National Research Centre, Dokki, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water.

2.7. In vivo antioxidant activity

Forty eight adult male albino rats were divided into eight groups, each of six animals, as follows:

First group; rats received 1 ml saline and kept as negative control. Second group; diabetic rats that kept untreated (positive control). Diabetes was induced according to the method described by Eliasson & Samet [13] using a single dose of intraperitoneal injection of 150 mg/kg b.wt. Alloxan, followed by an overnight fast. Third group; diabetic rats that received 7.5 mg/kg of vitamin E as a reference drug (positive control). Fourth, fifth, sixth, seventh and eighth groups; diabetic rats that received orally 100 mg/kg b.wt. of petroleum ether,

methylene chloride, ethyl acetate, butanol and aqueous extracts, respectively. The rats received the extract and the drug for seven days. At the end of the experiment, blood glutathione was estimated using biodiagnostic kits [14].

2.8. Acute anti-inflammatory activity

Paw swelling, or footpad oedema, is a convenient method for assessing acute anti-inflammatory activity [15]. This model uses carrageenan as an irritant to induce paw oedema. Typically, test materials are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, reduce paw swelling in a dose-dependent manner. Forty two adult male albino rats were divided into seven groups, each of six animals, as follows: First group; rats that received 1 ml saline serving as a control. Second, third, fourth, fifth and sixth groups; rats received 100 mg/kg b.wt. of petroleum ether, methylene chloride, ethyl acetate , butanol and aqueous extracts, respectively. Seventh group; rats that received 20 mg/kg b.wt. of the reference drug (indomethacin). One hour after oral administration, all animals were given a sub-plantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Four hours after drug administration, the rats were sacrificed. Both hind paws were, separately, excised and weighed. The percentage oedema was calculated according to the following equation:

% Oedema = (Wt. of right paw –Wt. of left paw) x 100/ Wt. of left paw

% Oedema inhibition = (Mc-Mt) x 100/ Mc,

Where**:** Mc **=** the mean oedema in control group, Mt **=** the mean oedema in the drug- treated group.

2.9. Analgesic activity

2.9.1. Hot plate test

Hot plate test technique was carried out as described by [16]. It was used to evaluate pain thresholds. Forty two rats were divided into seven groups. Each group contains 6 animals. First group; rats received 1 ml saline. Second, third, fourth, fifth and sixth groups; rats received orally 100 mg/kg b.wt. of petroleum ether, methylene chloride, ethyl acetate, butanol and aqueous extracts, respectively. Seventh group; rats that received 20 mg/kg b.wt. of the reference drug (indomethacin). Each rat was placed on the hot plate in order to obtain animal's response to electrical heat induced nociceptive pain stimulus. The latency (in seconds) is the time till the animals showed first signs of discomfort (hind paw lifting, hind pw licking or jumping) was taken as indicator of animals response. The % Protection against thermal pain stimulus was calculated according to the following equation:

% Protection against thermal pain stimulus = test mean-control ×100/ Control mean

2.9.2. Writhing test:

The analgesic activity was determined by acetic acid induced writhing method using Swiss male albino mice (20-25g). Animals were acclimatized to laboratory conditions for at least 1 hr before testing and were used once during the experiment. Forty two adult male albino mice were divided into seven groups, each of six animals, as follows: First group; mice that received 1 ml saline serving as a control. Second, third, fourth, fifth and sixth groups; mice received 100 mg/kg b.wt. of petroleum ether, methylene chloride, ethyl acetate, butanol and aqueous extracts, respectively. Seventh group; mice that received 4 mg/kg b.wt. of the standard analgesic (Indomethacin). Thirty min. later 0.6% acetic acid was injected intraperitoneal (0.2 ml/mice). Each mouse was then placed in an individual clear plastic observed chamber and the total no. of writhes/ 30 min. was counted for each mice [17]. The number of writhing and stretching was recorded and compared with the control drug. The percent was calculated using the following ratio:

% of protection = Control mean- treated mean ×100/Control mean.

2.10. Antiulcer activity

This effect was carried out according to Corell et al[18].Thirty six male albino rats of Sprague Dawley strain 130- 140 g were divided into six groups. All groups received oral indomethacin (20 mg/kg). First group; rats received indomethacin and kept as a positive control. Second, third, fourth, fifth and sixth groups; rats orally received indomethacin+100 mg/kg b.wt. of petroleum ether methylene chloride, ethyl acetate, butanol and aqueous extracts, respectively. They were starved for 18 h but given only water, then four h later, the animals were sacrificed and the stomach removed, fixed in 10% formalin dissected along the greater curvature and the number of ulcer were counted using magnifying lens. The percent was calculated using the following ratio: % of protection = Control mean- treated mean ×100/ Control mean.

3. Results and Discussion

3.1. Identification of the isolated compounds

The present work deals with the isolation of nine compounds on the bases of chromatographic techniques [12] (Fig.1). Their structure clarification was carried out through color reactions, R_f values, chemical investigations (mild and complete acid hydrolysis) and physical investigations (MS, UV and NMR) [19]. Further authentication was carried out by comparison of their spectroscopic data with previously published values. They were identified as:

4-hydroxy benzoic acid (1)

White crystalline powder, ^{1}H-NMR (300 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 7.09 (2H, d, *J*= 8 Hz, H-2, 6), 6.64 (2H, *d*, *J*= 8 Hz, H-3, 5).

Gallic acid (2)

Yellowish-white crystals, *Rf* = 0.6 (BAW) and 0. 54 (15% AcOH). UVλ_{max} nm: (MeOH) 283 nm. ^{1}H- NMR (500 MHz, DMSO-*d6*, δ, ppm) : 7.01(2H, s, H-2, H-6).

Luteolin (3).

Yellow fine crystals, *Rf* = 0.81 (BAW) and 0. 08 (15% AcOH). UVλ_{max} (nm): (MeOH) 269, 349; (+NaOMe) 268, 399; (+$AlCl_3$) 273,301sh, 413; (+$AlCl_3$/HCl) 268, 298, 357, 390; (+ NaOAc) 268, 356; (+NaOAc/H_3BO_3) 260, 370. ^{1}H- NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 12.98 (1H, *s*, 5-OH); 7.4 (1H , dd , *J* =2.0 Hz ,7.8 Hz, H-6´); 7.39 (1H, d, *J*= 1.5 Hz, H-2´); 6.87 (1H , d, *J* = 7.8 Hz, , H-5`); 6.67 (1H, s, H-3); 6.4 (1H , d, *J* = 2 Hz, H-8); 6.16 (1H, d, *J* = 2 Hz, H-6).

Myricetin (4).

Yellow powder, *Rf*= 0.48 (BAW) and 0. 02 (15% AcOH). UVλ_{max} (nm): (MeOH) 255, 300sh, 375; (+NaOMe): 278, 328 sh, 436; (+$AlCl_3$) 270, 314 sh, 460; (+$AlCl_3$/HCl) 269, 307 sh, 433; (+NaOAc) 262, 300 sh, 388; (+NaOAc/H_3BO_3) 257, 394. ^{1}H- NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 7.20 (2H, s, H-2', 6'); 6.32 (1H, d, *J*=2.5 Hz, H-8); 6.13 (1H, d, *J*=2.5Hz, H-6).

Luteolin 7-*O*-*β*-glucoside (5)

Yellow amorphous powder with *Rf*= 0.47 (BAW) and 0.5 (15% AcOH). UVλ_{max} nm: MeOH 256, 269 sh, 348 + NaOMe263, 403 + $AlCl_3$ 274, 298 sh, 335, 423 + $AlCl_3$ / HCl 274, 296 sh, 355, 387 + NaOAc 263, 355, 411 + NaOAc/H_3BO_3 261, 373. ^{1}H- NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 7.4 (2H,m,H-2', 6'); 6.8 (1H, d, *J*=8.5 Hz, H-5'); 6.73 (1H ,d, *J*= 2 Hz , H-8); 6.70 (1H ,s, H-3); 6.37(1H, d, *J*= 2 Hz , H-6); 5.05 (1H ,d, *J*= 7.4 , H-1").

Luteolin-7-*O*-*β*-rutinoside (6)

Yellow amorphous powder, *Rf*= 0.32 (BAW) and 0. 23 (15% AcOH). UVλ_{max} nm: (MeOH) 256, 267 sh, 351; (+NaOMe) 269, 402; (+$AlCl_3$) 271, 298 sh, 328, 428; (+$AlCl_3$/ HCl) 262, 298 sh, 357, 392; (+NaOAc) 263, 355sh, 411; (+NaOAc/H_3BO_3) 261, 373. ^{1}H-NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 7.43 (1H, dd, *J*= 2 Hz, 8.1 Hz, H-6'), 7.39 (1H ,d, *J*=2Hz, H-2'); 6.90 (1H , d, *J*= 8.1 Hz, H-5'); 6.75 (1H , d, *J*= 2 Hz, H-8); 6.73(1H, s, H-3); 6.44 (1H , d, *J*= 2 Hz, H-6); 5.03 (1H, d, *J*= 6.9 Hz, H-1"); 4.5 (1H , d, *J*= 2 Hz, H-1'"); δ 1.06 (3H , d, *J*= 6.3 Hz, H-6'"). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 182.01(C-4), 163.49 (C-2), 161.62 (C-7); 161.62 (C-5); 157.51(C-9), 149.65(C-4′);147.03 (C-3′), 127.77 (C-1′), 119.9 (C-6′),116.54 (C-5′), 113.32 (C-2′),105.89 (C-10), 102.80 (C-3), 100.6 (C-1″),100.6 (C-6), 100.27(C-1‴), 95.04 (C-8), 76.61 (C-3″), 76.60 (C-5″), 72.51 (C-4‴), 72.51 (C-2″) ,71.01 (C-3‴),70.71 (C-2‴), 70.06 (C-4″), 68.80 (C-5‴), 66.40 (C-6″), 17.92 (C-6‴).

Myricetin 3-*O*-*β*-glucoside (7)

yellowish brown amorphous powder with *Rf*= 0.32 (BAW) and 0. 42 (15% AcOH). UVλ_{max} nm: (MeOH) 260, 353; (+NaOMe) 272, 322, 389; (+$AlCl_3$) 271, 394; (+$AlCl_3$/HCl) 269, 305, 354, 397sh; (+NaOAc) 265, 356; (+NaOAc/H_3BO_3) 261, 369. ^{1}H-NMR (300 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): δ 6.89 (2H, s, H-2', 6'); 6.37 (1H, d, *J*=2, H-8); 6.02 (1H, d, *J*=2, H-6); 5.2 (1H, d, *J*=6.9 Hz, H1").

Myricetin 3-*O*-α-rhamnoside (8)

Yellowish brown amorphous powder with *Rf*= 0.4 (BAW) and 0.53 (15% AcOH). UVλ_{max} nm: (MeOH) 259, 357; (+ NaOMe) 269, 405; (+$AlCl_3$) 270, 340sh, 425; (+$AlCl_3$/HCl) 271, 364sh, 403; (+NaOAc) 266, 394; (+NaOAc/H_3BO_3) 261, 374. ^{1}H- NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 12.64 (1H, s , 5-OH); 6.84 (2H, s,

H-2', 6'); 6.32 (1H, d, *J*=2 Hz, H-8); 6.15 (1H, d, *J*=2 , H-6); 5.15 (1H , d, *J*=1.2 Hz, H1") ; 0.80 (3H, d, *J*=6 Hz H6").

Velutin (5, 4´dihydro-3′, 7 dimethoxy-flavone) (9)

Yellow amorphous powder, *Rf*= 0.72 (BAW) and 0.03 (15% AcOH). UVλmax nm: (MeOH) 273, 331; (+ NaOMe) 283, 326 sh, 400; (+$AlCl_3$) 280, 305, 346; (+$AlCl_3$/HCl) 279, 305, 344; (+NaOAc) 282, 371; (+NaOAc/H_3BO_3) 276, 320sh, 352. ^{1}H-NMR (500 MHz, DMSO-*d6*, δ, ppm, *J*/Hz): 8.3(1H ,d, *J*= 2 Hz, H-2'); 7.7(1H ,dd , *J*= 8 Hz, 2 Hz, H-6'); 6.67(2H ,bs, H-3,8); 6.8 (1H ,d, *J*= 8 Hz, H-5'); 6.3(1H ,d, *J*= 2 Hz H-6); 4.2(3H , s, OCH_3 at position 3'), 4.1(3H, s, OCH_3 at position 7).

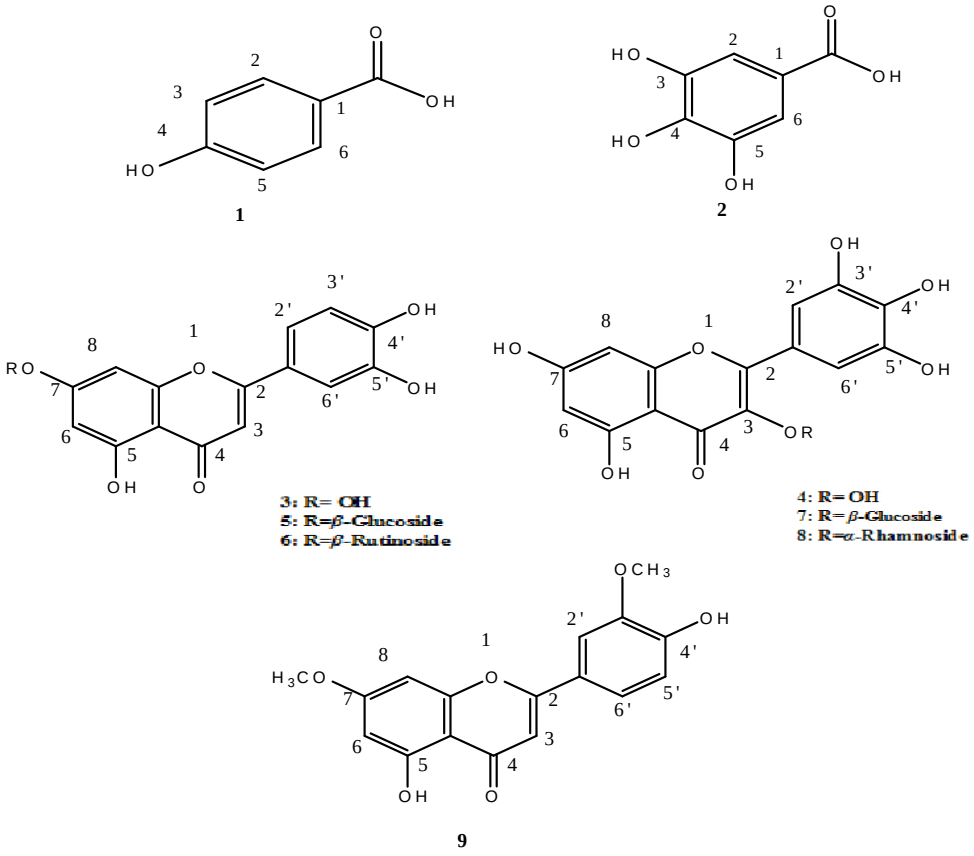

Fig.(1): Chemical structures of isolated and identified compounds of *A. tomentosus*

3.2. In vivo antioxidant activity evaluation of successive leaf extracts compared to vitamin E

Methylene chloride, ethyl acetate and butanol extracts showed significant antioxidant activity with % of change from control; 61.75%, 59.44% and 52.53 %, respectively. Moreover, the petroleum ether and aqueous extracts showed weak activity with 30.87% for each (Table 1). The relative potencies of methylene chloride, ethyl acetate, butanol, aqueous and petroleum ether extracts were 94.37%, 90.84%, 80.2%, 47.18% extract and 47.18 % , respectively, as compared with vitamin E (Table 1).

Table (1): Antioxidant effect of successive extracts of *A. tomentosus* leaves

Animal group (Diabetic rats)	Dose(mg/kg/b.wt.)	Blood Glutathione (% mg)	% of change from control	% of potency
Diabetic rats		-	-	-
PEE	100	28.4±0.6*	30.87	47.18
MCE	100	35.1 ± 1.3*	61.75	94.37
EAE	100	34.6 ± 1.1*	59.44	90.84
BE	100	33.1±0.4*	52.53	80.28
AE	100	28.4 ± 0.6*	30.87	47.18
Vitamin E (control)	7.5	35.9 ± 0.9*	65.43	100

PEE; Petroleum ether extract, MCE; Methylene chloride extract, EAE; Ethyl acetate extract, BE; Butanol extract, AE; Aqueous extract, *significantly different from control group at P<0.01.

The significant antioxidant activity of the methylene chloride, ethyl acetate and butanol extracts may be due to the presence of flavonoids. The Preclinical studies have shown that the flavonoid such as luteolin and its glycosides are widely distributed in the plant kingdom and significantly this flavone possesses a variety of pharmacological activities, including antioxidant activity [20].

3.3. Acute anti-inflammatory activity

A significant anti-inflammatory activity was exhibited by all extracts as indicated by inhibition of the rat paw oedema weight induced by carrageenan. The highest activity exhibited by methylene chloride (87.57%), followed by aqueous (80.66%), ethyl acetate (62.42 %), petroleum ether (45.78%) then butanol extract (34.59%), in comparison with indomethacin (100 % potency) (Table 2).

The activity of the more polar extracts may be due to their flavonoidal constituents. Luteolin may be a potent selective inhibitor of COX-2 and the inhibition is attributable to its down-regulation of the mRNA expression of COX-2 in inflammatory responses [21].

Table (2): Acute anti-inflammatory activity of the successive extracts of *A. tomentosus* leaves

Animal group (Rats)	Dose (mg/kg/b.wt.)	% Paw Oedema		% of Potency
		Mean ± S.E	% Inhibition	
Control	1 ml saline	59.4±1.8	-	-
PEE	100	42.1±1.6*	29.12	45.78
MCE	100	26.3±0.7*	55.7	87.57
EAE	100	35.8±1.3*	39.7	62.42
BE	100	46.2±1.6 *	22.0	34.59
AE	100	28.9±0.9*	51.3	80.66
Indomethacin 20mg/kg b.wt.	20	21.6±0.7*	63.6	100

PEE; Petroleum ether extract, MCE; Methylene chloride extract, EAE; Ethyl acetate extract, BE; Butanol extract, AE; Aqueous extract, *significantly different from control group at P< 0.01.

3.4. Evaluation of analgesic Activity (writhing test and hot plate test)

A significant analgesic activity was exhibited by all extracts as indicated by the delay in signs of discomfort (hind paw lifting, hind paw licking or jumping) and decreases in total no. of writhes/30 min. In Writhing test, the highest analgesic activity was exhibited by methylene chloride (70.96%), followed by aqueous (61.49%), ethyl acetate (57.69%), butanol extract (40.20%), and petroleum ether (28.31%), in comparison with indomethacin (100 % potency) (Table 3).

Table (3): Analgesic activity of successive extracts of *A. tomentosus* leaves (Writhing test):

Animal group (Mice)	Dose (mg/kg.b.wt.)	No. of abdominal writhing constrictions	% of inhibition	% of Potency
Control	1ml saline	46.7 ± 1.3	-	-
PEE	100	38.6 ±1.3*	17.34	28.31
MCE	100	26.4 ± 0.5*	43.46	70.96
EAE	100	30.2 ± 0.9*	35.33	57.69
BE	100	35.2 ± 1.1*	24.62	40.20
AE	100	29.1± 0.8*	37.66	61.49
Indomethacin	20	18.1±0.3*	61.24	100

PEE; Petroleum ether extract, MCE; Methylene chloride extract, EAE; Ethyl acetate extract, BE; Butanol extract, AE; Aqueous extract, *significantly different from control group at P< 0.01.

In hot plate test, the highest analgesic activity was exhibited by methylene chloride (78.46%), followed by ethyl acetate (59.41%), aqueous (54.94%), butanol extract (17.69%) and petroleum ether (10.8%), in comparison

with indomethacin (100 % potency) (Table 4). The high analgesic activity of methylene chloride and ethyl acetate may be due to the presence of luteolin which has strong antinociceptive (analgesic) properties, sometimes more active than well-known analgesic drugs, such as acetyl salicylic acid, acetaminophen, dipyrone, indomethacin [22]. Myricetin inhibited the content of PGE2 in the peritoneal fluid and platelet aggregation induced by collagen and arachidonic acid in vitro. These results collectively demonstrated that myricetin possessed potent analgesic activity, which was related with peripheral analgesia, but, not with the opioid system. Myricetin may be a potent COX-1 inhibitor with anti-platelet activity [23].

Table (4): Analgesic activity (Hot plate test) of successive extracts of *A. tomentosus* leaves:

Animal group (Rats)	Dose (mg/kg.b.wt.)	Prehot plate Latency (sec.)	Post exposure hot plate latency (sec.)	% of change	% of Potency
Control	1ml saline	12.4±0.7	13.1±0.6	5.645	6.260
PEE	100	12.1±0.5	13.2±0.7	9.090	10.08
MCE	100	10.6±0.4	18.1± 0.7*	70.75	78.46
EAE	100	11.2±0.3	17.2 ± 0.9*	53.57	59.41
BE	100	11.9±0.6	13.8 ± 0.7	15.96	17.69
AE	100	10.9±0.4	16.3± 1.1*	49.54	54.94
Indomethacin	20	11.2±0.8	21.3±1.4*	90.17	100

PEE; Petroleum ether extract, MCE; Methylene chloride extract, EAE; Ethyl acetate extract, BE; Butanol extract, AE; Aqueous extract, *significantly different from control group at P< 0.01.

3.5. Antiulcer activity of successive leaf extracts of A. tomentosus leaves

A significant anti-ulcer activity was exhibited by all extracts as indicated by examining any eroded or ulcerated areas with magnifying lens.

In Corell test, the highest anti-ulcer activity was exhibited by methylene chloride (65.85%), followed by ethyl acetate (56.09%), aqueous (48.0%), butanol extract (38.21%) and pet. ether (22.76%), (Table 5). The high antiulcer activity of methylene chloride and ethyl acetate may be due to the presence of phenolic constituents. Flavonoids and terpenoides display anti-secretory and cytoprotective properties in different experimental models of gastric ulcers [24]. Moreover, gallic acid possesses antiulcer effect and that these occur by a mechanism that involves attenuation of offensive factors, improvement of mucosal defensive with activation of antioxidant parameters and inhibition of some toxic oxidant parameters [25].

Table (5): Antiulcer activity of successive extracts of successive extracts of *A. tomentosus* leaves:

Animal group	Dose(mg/kg.b.wt.)	NO. of gastric ulcers	% of Protection
Indomethacin (Control)	20	12.3±0.6	-
Indomethacin+ PEE	20+100	9.5±0.4*	22.76
Indomethacin+ MCE	20+100	4.2±0.1*	65.85
Indomethacin+ EAE	20+100	5.4±0.3*	56.09
Indomethacin+ BE	20+100	7.6±0.5*	38.21
Indomethacin+ AE	20+100	6.3±0.3*	48.0

PEE; Petroleum ether extract, MCE; Methylene chloride extract, EAE; Ethyl acetate extract, BE; Butanol extract, AE; Aqueous extract, *significantly different from control group at P< 0.01.

Conclusion

The methylene chloride, ethyl acetate and aqueous extracts of *A. tomentosus* leaves have a potent effects as anti-inflammatory, analgesic, antiulcer and antioxidant activity. These effects could be attributed to a synergistic effect of the phenolic compounds. Thus, they could be used as supplement in dosage forms. This requires the development of phytopharmaceuticals containing the standardized bioactive ingredients after applying clinical studies.

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