Journal of Medicinal Plant Research

Volume 10 Number 16, 25 April, 2016
ISSN 1996-0875

Academic Journals

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Journal of Medicinal Plants Research

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Vol. 10(16), pp. 209-215, 25 April, 2016
DOI: 10.5897/JMPR2015.6031
Article Number: 379205E58339
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Chemical composition and biological activities of *Pouteria campechiana* (Kunth) Baehni

Aly M. Elsayed[1], Nebal D. El-Tanbouly[1], Sherifa F. Moustafa[1*], Rabab M. Abdou[1] and Sally A. W. El Awdan[2]

[1]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, P. B. 11562 Cairo, Egypt.
[2]Pharmacology Department, National Research Center, Tahrir Street, Dokki, P. B. 12622 Cairo, Egypt.

Received 13 December, 2015; Accepted 3 March, 2016

The present study was conducted to evaluate the analgesic, anti-inflammatory and gastroprotective activities of ethanol, de-fatted ethanol extracts and n-hexane fractions of *Pouteria campechiana* (Kunth) Baehni leaves and seeds. Further chemical analysis was done to isolate and identify its bioactive compounds. The seeds ethanolic extract produced 85% inhibition of inflammation in the rat paw oedema test at the dose of 100 mg/kg after 4 h ($p<0.05$). On the other hand, the leaves ethanolic extract (200 mg/kg) exhibited maximum analgesic activity after 90 min ($p<0.05$) in the hot plate test. Both leaves and seeds ethanolic extracts showed significant decreases in gastric ulcer number and severity ($p<0.05$). Phytochemical investigation of *P. campechiana* leaves and seeds yielded six compounds: protocatechuic acid (C_1), gallic acid (C_2), quercetin (C_3), myricetin (C_4), myricetin-3-*O*-*α*-L-rhamnoside (C_5) and myricetin-3-*O*-*β*-galactoside (C_6). The study supports the use of *P. campechiana* in traditional medicine for conditions associated with inflammation, pain and peptic ulcers.

Key words: *Pouteria campechiana,* anti-inflammatory, analgesic, gastric ulcer, flavonoids, phenolics.

INTRODUCTION

The genus *Pouteria* belongs to the family Sapotaceae which is widely found around the world. Several *Pouteria* species have been used in folk medicine to treat inflammation, skin eruptions, ulcers and back pain (Silva et al., 2009). However, many of them lack scientific evidences about these activities that is, *Pouteria campechiana* (Kunth) Baehni seeds which was used in folk medicine as a remedy for ulcers (Morton, 1987). *P. campechiana* is valued as an ornamental tree and its wood has found commercial application for buildings construction. In its native range, it has been a source of latex used to adulterate gum (Morton, 1987). A decoction of *P. campechiana* bark is taken as a febrifuge in Mexico and is applied on skin eruptions in Cuba. Six stilbenoids and six flavonoid glycosides were previously identified from ethyl acetate extract of *P. campechiana* leaves and their antimitotic activities were evaluated (Hernandez et al., 2008).

The fruit of *P. campechiana* (Kunth) Baehni is reported as a rich source for carotenoids (Costa et al., 2010) and is well known as an antioxidant and a hepatoprotective (Kubola et al., 2010; Aseervatham et al., 2013).

*Corresponding author. E-mail: sherifa.mostafa@pharma.cu.edu.eg.

Long term use of synthetic anti-inflammatory and/ or analgesic drugs is associated with numerous side effects including stomach bleeding and ulcers, high blood pressure and kidney problems (Maroon et al., 2010). Nevertheless, there are many natural products that exhibit anti-inflammatory and analgesic properties and have relatively low incidences of side effects. Production of safe and potent analgesic, anti-inflammatory and anti-ulcerogenic drugs from natural origins has been recently investigated.

To the best of our knowledge no reports were found about the anti-inflammatory, analgesic, antiulcerogenic activities of *P. campechiana* leaves and seeds. This study was therefore designed to evaluate the aforementioned activities and to identify the biologically active compounds.

MATERIALS AND METHODS

Plant

Plant material of *P. campechiana* (Kunth) Baehni was collected in December 2010 from El-Mansouria, Giza Governorate, Egypt and was kindly identified by Shahina A. Ghazanfar, Head of Temperate Regional Team, Royal Botanic Gardens Kew, UK and Dr. Mohammed El-Gibali former senior botanist at National Research Center, Cairo, Egypt. A voucher specimen (No. 19.4.2015) was deposited at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Preparation of plant extracts

Air dried powdered leaves (1.3 kg) and seeds (900 gm) were separately macerated in 70% ethyl alcohol till exhaustion. The ethanol extracts were filtered and evaporated under reduced pressure, at a temperature not exceeding 50°C, to yield 145 g of solid dark green residue of the leaves ethanolic extract and 67 g semisolid brown residue of the seeds extract. The residue of the ethanol (70%) extract of the seeds and leaves were separately suspended in distilled water and partitioned with *n*-hexane. . The de-fatted ethanol and n-hexane fractions were kept for the pharmacological study. The ethanolic extracts were tested for anti-inflammatory, analgesic and antiulcerogenic activities. Furthermore, the fractions of the most active ethanol extract were tested for the aforementioned activities. The de-fatted ethanol fractions were further partitioned successively with methylene chloride, ethyl acetate and n-butanol for the phytochemical study.

Animal bioassay

Adult male albino Wistar rats (aged 8 weeks) weighing 120 to 150 g were used for the experiments. Animals were randomized into groups (n=6-8). All the animals were obtained from the animal house colony of the National Research Center (Giza, Egypt). The animals were maintained under standard environmental conditions and fed with standard diet and water *ad libitum.* Animal usage in the experiments was approved by Research Ethics Committee, Faculty of Pharmacy, Cairo University in Egypt (approval number MP 13). A Preliminary pharmacological screening for seeds and leaves total alcohol extracts for each pharmacological effect (anti-inflammatory, analgesic and anti-ulcerogenic) was done. The extract with the best pharmacological results in each activity was further fractionated into polar and non-polar fractions (n-Hexane and de-fatted alcohol) and further tested for the previous effects. According to this scheme the study proceeded.

Experimental models

Median lethal dose LD_{50}

The LD_{50} values were calculated (Karber, 1931). Preliminary experiments were done to determine the minimal dose that killed all animals (LD_{100}) and the maximum dose that failed to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses; each dose was administrated orally in a group of 6 rats. The rats were then observed for 24 h and symptoms of toxicity and mortality in each group were recorded.

Carrageenan-induced rat paw oedema method

Procedures described by Winter et al. (1962) were used for evaluating anti-inflammatory activity. The rats were divided into 14 groups (n = 6). Group I, received saline 0.9% (control); Group II, indomethacin (20 mg Kg^{-1}) serving as a positive control and the other twelve groups were treated with ethanol extract, defatted ethanol fraction and hexane fraction of the leaves and seeds each at two doses 100 and 200 mg kg^{-1}. Oedema was induced in the right hind paw of each rat by sub plantar injection of 100 µl of 1% carrageenan. The plant extracts were administered orally 1 h before induction of inflammation. Paw volume of both control and plant extract treated rats were measured at 1, 2, 3, and 4 h after carrageenan injection by water displacement method (Chattopadhyay et al*.,* 2002; Li et al., 2003). The percentage of oedema inhibition in treated animals versus control was calculated.

Hot plate method

Hot plate method was carried out according to procedures of Woolfe and McDonald (1944). The rats were divided into ten groups (n = 8). Saline was administered (s.c.) in Group I as a control. Indomethacin was administered (20 mg kg^{-1}) in Group II as a positive control and the other eight groups were given leaves and seeds ethanol extracts, leaves defatted ethanol fraction and hexane fraction of the leaves each at two doses 100 and 200 mg kg^{-1}. The reaction time between the moment the rat reached the plate and that when the animal licked its paw was measured after 0, 30, 60 and 90 min following the administration of the tested plant extracts or saline. Analgesic activity was expressed by the prolongation of the reaction time.

Ethanol-induced ulcer model

Acute erosion of the gastric mucosa was induced in fasting rats by intragastric administration of absolute ethanol (Robert et al., 1979). The rats were divided into 14 groups (n = 6). Ranitidine (20 mg/kg, 60 min prior to ethanol) was used in one group as a reference drug; the control group was given ethanol and the other twelve groups were given ethanol extracts, defatted ethanol fractions and hexane fractions of the leaves and seeds, each at two doses 100 and 200 mg kg^{-1}. The animals were sacrificed 1 h after being given ethanol and the gastric lesions were examined under an illuminated magnifier (Adami et al., 1964).

Isolation of bioactive constituents

A weighed amount (3 g) of the ethyl acetate fraction of *P.*

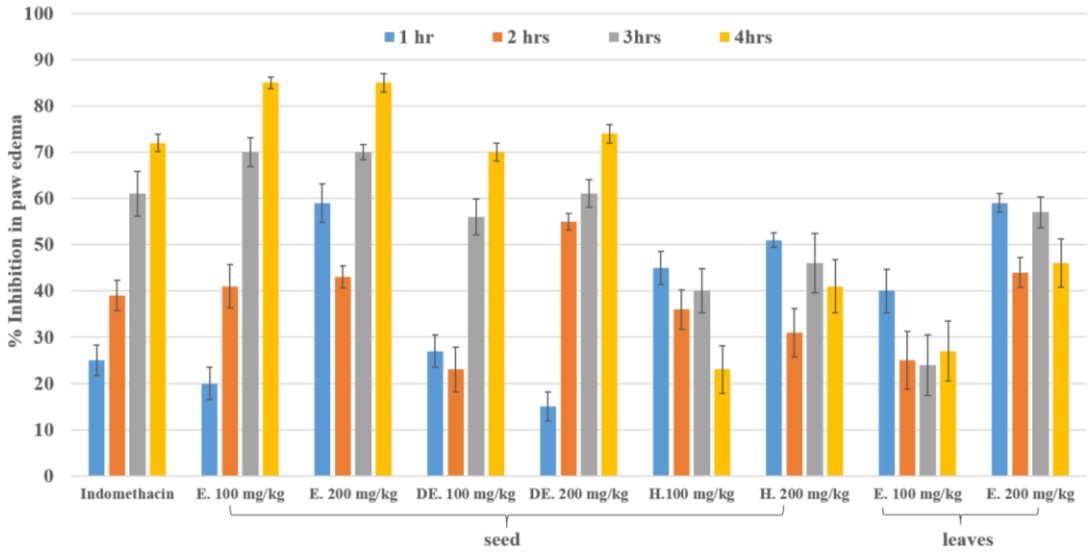

Figure 1. Histogram presenting anti-inflammatory activity of *P. campechiana* seeds and leaves extracts on carrageenan induced hind paw oedema method in relation to different doses. E (ethanol extract), DE (de-fatted ethanol fraction), H (*n*-hexane fraction). * Statistically significant from the control normal group at the corresponding time: $p< 0.05$.

campechiana seeds was subjected to chromatography using Sephadex LH-20 column. Isocratic elution was performed with methanol:water (90:10 v/v) collecting fractions of (20 ml). Sub fraction (18 to 21) was re-chromatographed on Silica gel 60 column using chloroform:methanol:water (98:2:0.5 v/v) as eluting system to get 30 mg of compounds C_1 and 45 mg of compound C_2.

A weighed amount (10 g) of the ethyl acetate fraction of *P. campechiana* leaves was subjected to fractionation by vacuum liquid chromatography (VLC) on 100 g silica gel H. Gradient elution was performed using chloroform/ethyl acetate mixtures, ethyl acetate and ethyl acetate/methanol mixtures. The polarity was increased by 10% till 100% methanol was reached. Collective fractions I (60% Ethyl acetate in chloroform) was subjected to re-chromatographing using Sephadex LH-20 column; isocratic elution was performed using methanol. Pooled fractions (450 mg) were further purified using Sephadex LH-20 column and eluted with water-methanol (50:50 v/v). An impure fraction showing one major spot was obtained and re-chromatographed on Sephadex LH-20 using methanol:water (90:10 v/v) as an eluting system to get 30 mg of compound C_3. Fraction II (70% Ethyl acetate in chloroform) was subjected to re-chromatography on Sephadex LH-20 column; isocratic elution was performed with methanol. Pooled fractions (220 mg) were further purified using Sephadex LH-20 column; eluted with water-methanol (50:50 v/v) yielding 35 mg yellow powder of compound C_4. Fraction III (10% Methanol in ethyl acetate) was subjected to further re-chromatographing on Silica gel 60 column; elution was started using chloroform and the polarity was increased using methanol. Pooled fractions yielded upon concentration under vacuum 45 mg of compound C_5. Fraction IV (20 to 40% Methanol in ethyl acetate) was subjected to re-chromatographing using polyamide column and gradient elution with water:methanol. Pooled fractions (200 mg) were further purified using Sephadex LH-20 column; eluted with chloroform:methanol (50:50 v/v) and yielding 18 mg of compound C_6.

Statistical analysis

Statistical analysis was carried out using repeated measures one way analysis of variance (ANOVA) followed by least significant test for multiple comparisons. Effects are considered significant at $p< 0.05$. Results are expressed as mean ± standard error.

RESULTS AND DISCUSSION

The LD_{50} of the leaves (2.5 g/kg) and seeds (2 g/kg) *P. campechiana* ethanolic extracts are of a relatively high safety margin at the tested dose levels (Bucket al., 1976).

Acute anti-inflammatory effect was proved by the percentage of oedema inhibition in this study. The seeds ethanolic extract was significantly ($p<0.05$) more active (85%) than the leaves ethanolic extract (46%) at a dose of 200 mg/kg after 4 h (Figure 1). It was more effective in inhibiting oedema volume when compared with indomethacin as a standard. The seeds defatted ethanol fraction at doses of 100 and 200 mg/kg and reduced oedema by 70 and 74%, respectively. While *n*-hexane fraction at doses of 100 and 200 mg/kg reduced oedema by 32 and 41%, respectively, compared to its original volume. Both extracts did not cause gastric ulcers as usually expected with anti-inflammatory drugs.

Increasing doses of *P. campechiana* leaves and seed ethanolic extracts (100 and 200 mg/kg) significantly exhibited potent analgesic activity (Figure 2). The leaves ethanolic extract was more potent than that of the seeds at a dose of 200 mg/kg after 90 min. At 90 min, responses at dose 100 and 200 mg/kg were 8.7 and 8.44 min, respectively, for leaves' defatted ethanol fraction, 7.6 and 6.58 min for *n*-hexane fraction, respectively. The results revealed that the defatted ethanol fraction has a more potent analgesic activity compared with the *n*-hexane fraction consideration.

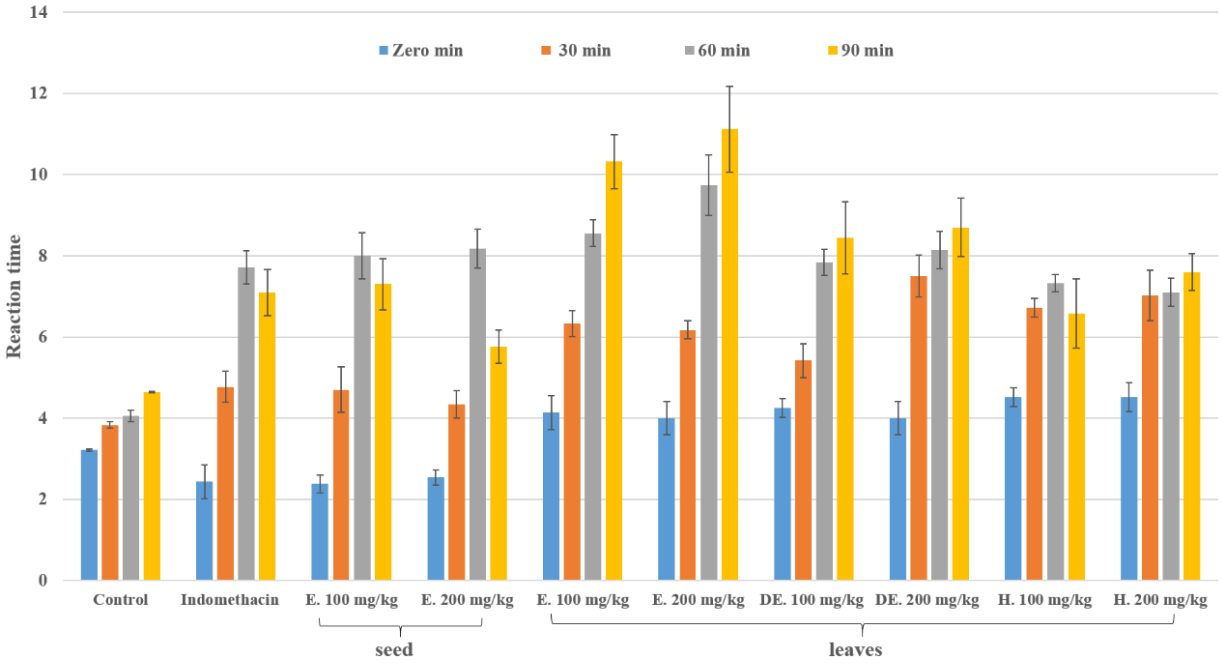

Figure 2. Histogram presenting the effect of *P. campechiana* seeds and leaves extracts in hot plate method in relation to different doses. E (ethanol extract), DE (de-fatted ethanol fraction), H (*n*-hexane fraction). * Statistically significant from the control normal group at the corresponding time: p< 0.05.

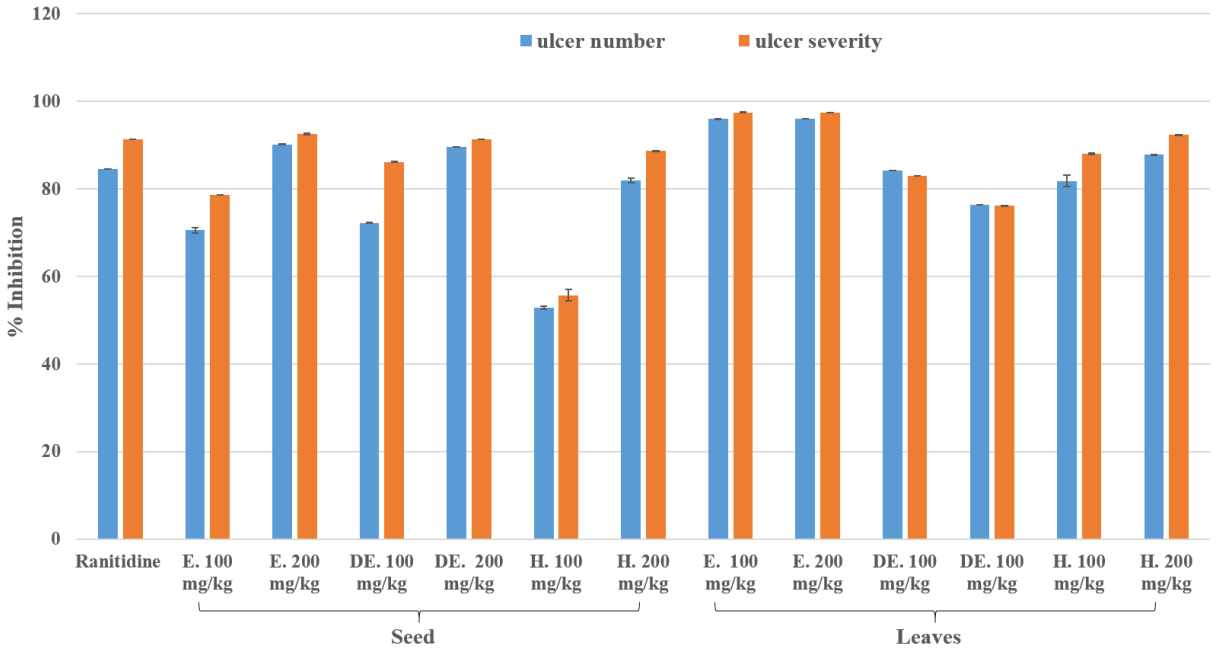

Figure 3. Histogram presenting antiulcer activity of different *P. campechiana* seeds and leaves extracts in relation to different doses. E. (ethanol extract), DE. (de-fatted ethanol fraction), H. (*n*-hexane fraction). * Statistically significant from the control normal group at the corresponding time: p< 0.05.

The different extracts and fractions at doses 100 and 200 mg/kg showed antiulcerogenic effects comparable with Ranitidine (50 mg/kg; p<0.05) (Figure 3). The most active, in this respect, was the leaf ethanolic extract

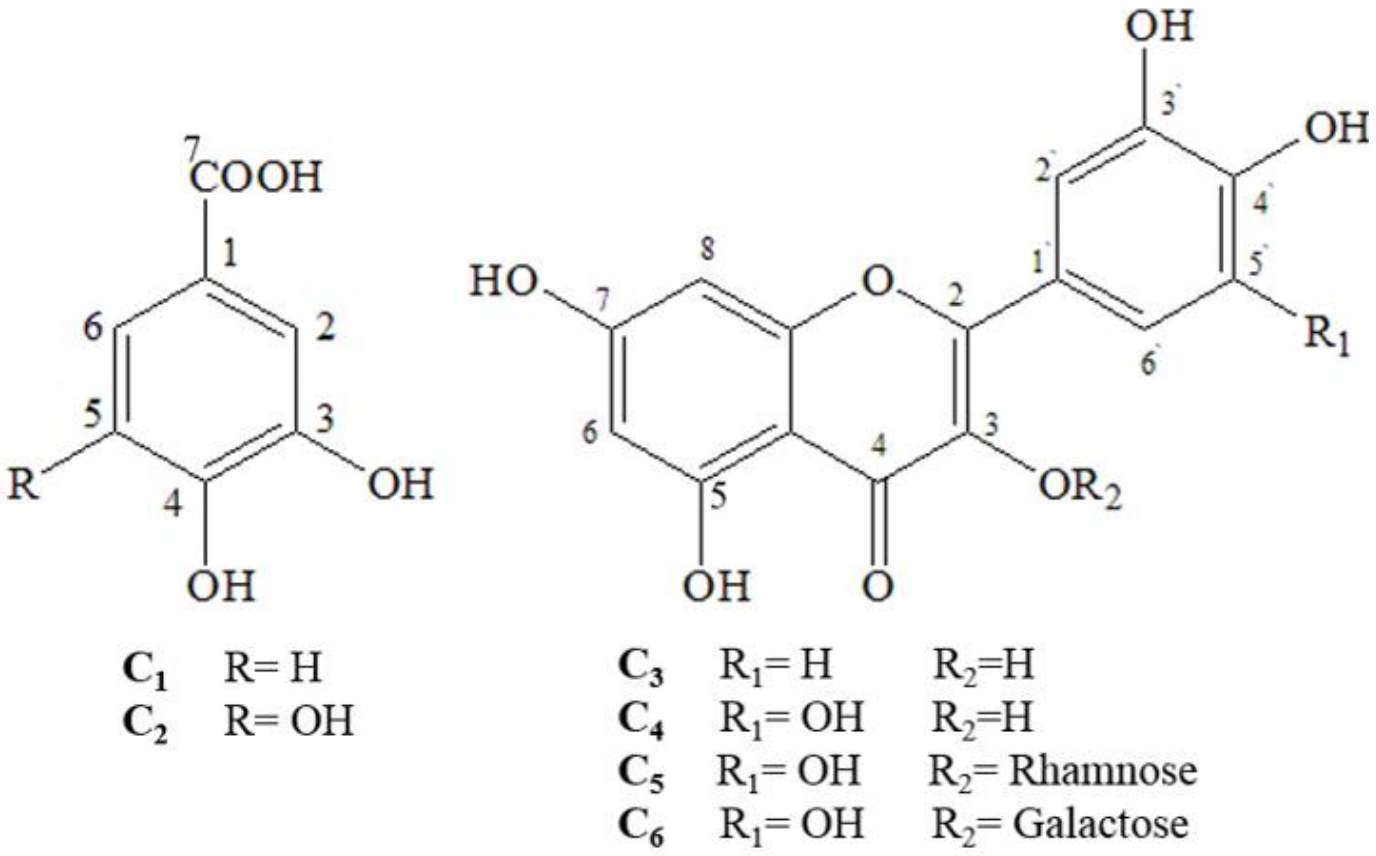

Figure 4. Structure of isolated compounds (**C_1-C_6**).

followed by the seed ethanolic extract at dose 200 mg/kg. The leaves ethanolic extract reduced ulcers number and severity by 95.98 and 97.44%, respectively. Ulceration of the gastric mucosa is known to be one of the main significant side effects produced of synthetic anti-inflammatory and/or analgesic drugs (Modi et al., 2012). Hence, it would be advantageous for a drug possessing anti-inflammatory activity and analgesic properties to have no ulcerogenic effect on gastric mucosa.

Unlike indomethacin, *P. campechiana* extracts and fractions have anti-inflammatory, analgesic drugs and anti-ulcer activities which could be explained by the combined effect of *P. campechiana* constituents.

There is abundant literature regarding medicinal plants establishing a relation between anti-inflammatory and analgesic activities and their phenol/flavonoid content (Deliorman et al., 2007; Saeed et al., 2010; Deng et al., 2011). From the results of our pharmacological study that showed higher activity for the de-fatted ethanol extracts and the reported literature linking these effects to the flavonoidal and phenolic contents, the authors were interested in identifying these content.

Six compounds were isolated from *P. campechiana* seeds and leaves (Figure 4). The compounds were identified through co-thin layer chromatography (TLC) comparison with authentic reference samples, and comparison of ^{1}H-NMR (Table 1) and ^{13}C-NMR (Table 2) with previously reported data. Compounds C_1 to C_6 were identified as protocatechuic acid (Yu et al., 2006), gallic acid (Kamatham et al., 2015), quercitin (Agrawal, 1989), myrcitin (Agrawal, 1989), myricetin-3-*O*-*α*-L-rhamnoside (Addae-Mensah and Achenbach, 1985) and myricetin-3-*O*-*β*-galactoside (Gürbüz et al., 2015), respectively. Protocatechuic acid was found to have promising anti-inflammatory and analgesic activity (Lende et al., 2011). Analgesic effects have previously been established for quercetin (Filho et al., 2008), myricetin (Tong et al., 2009) and myricetin-3-*O*-*β*-galactoside (Campos et al., 2013). Previous studies also link antiulcerogenic effect of medicinal plants to their flavonoids and phenolic contents (Sumbul et al., 2011).

From all of the aforementioned, it can be suggested that the analgesic, anti-inflammatory and antiulcerogenic effects found for *P. campechiana* in this study, may be attributed to its phenolic and flavonoid content.

Conclusion

The study proved that *P. campechiana* seeds and leaves

Table 1. ^{1}H-NMR chemical shifts (δ in ppm) for **C_1- C_4** (CD_3OD, 400MHz, *J* in HZ), **C_5**(DMSO, 300MHz, *J* in HZ), **C_6**(DMSO, 600MHz, *J* in HZ).

Proton No.	C_1	C_2	C_3	C_4	C_5	C_6
2	7.33 d,(*J*=1.9)	6.92 s	-	-	-	-
5	6.78 d, (*J*=8.2)	-				
6	7.28 dd, (*J*=8.2, 1.9)	6.92 s	6.17 d,(*J*=1.8)	6.08 d,(*J*=2)	6.20 d,(*J*=2.1)	6.16 br.s
8	-	-	6.36 d,(*J*=1.8)	6.28 d,(*J*=2)	6.37 d,(*J*=2.1)	6.35 br.s
2'	-	-	7.75 d,(*J*=1.8)	7.25 s	6.95 s	7.18 s
5'	-	-	6.89 d,(*J*=8.5)	-	-	-
6'	-	-	7.65 dd,(*J*=8.5, 1.8)	7.25 s	6.95 s	7.18 s
1''	-	-		-	5.32 d,(*J*=1.5)	5.32 d,(*J*=7.8)
2''	-	-		-	4.22 m	3.59 m
3''	-	-		-	3.79 dd, (*J*=3.3, 9.3)	3.42 m
4''	-	-		-	3.34 m	3.62 m
5''	-	-		-	3.51 m	3.37 m
6''	-	-		-	0.96 d,(*J*=6.3)	3.49 m

Table 2. ^{13}C-NMR chemical shifts (δ in ppm) for **C_1- C_2** (CD_3OD, 100 MHz), **C_5** (DMSO, 75 MHz), **C_6**(DMSO, 150 MHz).

Carbon No.	C_1	C_2	C_5	C_6
1	123.52	120.93	-	-
2	116.86	109.18	159.54	159.15
3	146.32	145.87	136.43	133.7
4	151.75	138.43	186.90	177.4
5	115.5	145.87	163.33	161.26
6	122.08	109.18	99.92	98.86
7	168.69	167.94	166.04	167.13
8	-	-	94.80	93.55
9	-	-	158.62	156.3
10	-	-	107.31	103.8
1'	-	-	122.02	121.54
2'	-	-	109.67	108.57
3'	-	-	146.96	145.5
4'	-	-	137.801	138.34
5'	-	-	146.96	145.5
6'	-	-	109.67	108.57
1''	-	-	103.76	102.12
2''	-	-	72.17	73.35
3''	-	-	72.24	75.99
4''	-	-	73.48	69.85
5''	-	-	72.02	77.98
6''	-	-	17.82	62.84

not only exhibited a potent anti-inflammatory and analgesic activity without forming gastric ulcers as expected with synthetic non-steroidal anti-inflammatory drugs, but also had a powerful antiulcerogenic activity. Tracing the available literature, it has been reported that flavonoids and phenolics can contribute to the anti-inflammatory, analgesic and anti-ulcerogenic effects. The authors were interested in identifying the phenolic and flavonoidal compounds in the ethanolic extracts and were able to isolate protocatechuic acid, quercetin, myricetin, and myricetin-3-O-β-galactoside, which were isolated for the first time from *P. campechiana*, besides gallic acid

and myricetin-3-O-α-L-rhamnoside which were previously isolated from *P. campechiana* fruits and leaves, respectively.

Conflict of Interests

The authors have not declared any conflict of interest exists.

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