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Isolation and Characterization of Bioactive Anti-inflammatory Flavonoids from *Arisaema tortuosum* Tubers

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Present study was focused on the isolation and characterization of the anti-inflammatory compound(s) from the methanolic extracts of *Arisaema tortuosum* tuber by column chromatography, TLC, FT-IR, ^{1}H, ^{13}C NMR and HR-MS. Among all the fractions obtained *A. tortuosum* fraction 3 (PC-1) and fraction 5 (PC-2) showed significant *in vivo* anti-inflammatory activity. This study endorsed that the isolated bioactive compound PC-2 showed better anti-inflammatory activity at a dose of 25 mg/Kg BW. The results indicate that PC-2 and PC-1 methanolic extract of *A. tortuosum* tuber may be competing for the tent in the therapy of inflammatory ailments.

Keywords: *Arisaema tortuosum*, Anti-inflammatory, Flavonoids.

INTRODUCTION

Whipcord cobra lily (*Arisaema tortuosum* (Wall.) Schott) exists as a perennial herb that refers to the family Araceae frequently grown in countries of the Himalayan region, including India, Western China and Myanmar, which has many promising ethnomedicinal effects [1]. The rhizomes, tubers, fruits, leave and corm of the plant are extensively utilized ethnomedicinally throughout India [2-4]. It also has been detailed to reveal the pharmacological features such as antibacterial, antifungal, antioxidant, anti-inflammatory, antiproliferative, analgesic and anticancer activity [5-7].

Flavonoids are a category of metabolites furnished in the world. Covers about their large commercial bioactive advantages, including antiviral/bacterial, blood-glucose-lowering, anti-inflammatory, anticancer, cardioprotective, antiaging for a long time undergone considerable recognition and competently braced by many studies [8-11]. So far, beyond 9000 flavonoids have been reported [12], mainly from food additives including tomatoes, tea, red wine, onions and apples [13,15]. Flavonoids are often assembled as glycosylated or esterified forms, comprising of C6-C3-C6 rings, namely rings A and B connected by three-carbon-ring C. As stated into replacement motif disparity, flavonoids can consequently be categorized into a divergent sub-category, assuming that an exceptionally multiple ranges of derivatives. Flavonoids are classified in six sub-classes on their chemical structures *e.g*. flavonols, flavones, isoflavones, flavanones, flavan-3-ols and anthocyanidins.

Flavones are established plentiful in leaves, flowers and fruits, for instance, celery, parsley, red peppers, mint and ginkgo biloba. The abstracted flavones are tangeritin. Flavonols like fisetin, silymarin, quercetin, rutin, kaempferol, myricetin and isorhamnetin are omnipresent in foods like tomatoes, apples, grapes, saffron, onions, kale, lettuce, berries, tea and red wine. Flavanones are broadly available in every citrus fruit, which gives the resentful taste of the juice and its peel. Lemons, grapes and oranges are affluent sources of flavanones such as naringenin, hesperidin and eriodictyol. Isoflavones are distinctive and bear a resemblance to estrogen in form and consequently, are categorized as phytoestrogens. Flavan-3-ols also called dihydroflavonols, incorporate catechin, gallocatechin, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate and procyanidin. The extremely often related nutrients with the flavan-3-ol compounds are green and black tea and fruits like, peaches, bananas, blueberries, apples and pears [15].

Despite several bioactive features that have been chalked up to *Arisaema tortuosum*, the outcomes of this plant were extensively considered. Phytochemical analysis of Whipcord

cobra lily (*Arisaema tortuosum* (Wall.) Schott) tuber contains a variety of triterpenoids, alkaloids, sterols, carbohydrates and flavonoids [16]. Despite *Arisaema tortuosum* considered for *in vitro* and *in vivo* frameworks concerning to distinct biological interest, no additional assignment has been implemented on the *in vivo* anti-inflammatory activity of tuber extracts and isolated compounds.

Consequently, this study pointed to isolate bioactive anti-inflammatory compounds from *Arisaema tortuosum* based on its *in vivo* anti-inflammatory venture on carrageenan-generated rat paw edema.

EXPERIMENTAL

Plant collection and identification: The plant material was collected from Darjeeling district, India in the month of July, 2016. Taxonomically of the plant was identified and authenticated by the Botanical Survey of India, Shibpur, India. The voucher specimen (BCDAPT/Priyanka/2015-16/01) has been conserved in Pharmacology Research Laboratory, BCDA College of Pharmacy & Technology, Barasat, India for further references.

Preparation of plant extract: Shade dried tubers of *Arisaema tortuosum* were powdered with the help of a mechanical grinder. The powder was extracted with petroleum ether for 72 h to remove the fatty materials. Then marc was extracted with methanol by the maceration process. The extract was concentrated in a rotary vacuum evaporator and the dried extract was stored in desiccators for further use [17,18].

Phytochemical test: The extract was put through qualitative tests for the perception of phytoconstituents existing in it namely with standard protocols [19].

Animals: The animals were grouped in the cage and kept in an air-conditioned room at 22 ± 1 ºC with a 12 h light and dark cycle. The animals were kept with normal pellet diet and water *ad libitum*. Following the ethical rules on animal experimentation; the research was conducted following the ethical rules on animal experimentation, approved by the Institutional Animal Ethics Committee (Approval No: 1682/PO/a/13/CPCSEA).

Isolation of compounds from methanolic extracts: By shaking with petroleum ether as first eluent silica gel 100-200 mesh was assembled into a homogenous suspension. The bottom part of the column was corked with a little cotton to avert the adsorbent pass out and then the silica gel suspension was streamed into the column, set aside for 10 min and used. The column 120 cm in length and 15 mm in diameter were washed with a suitable solvent and dried. The dried column was filled with petroleum ether up to two-third of the column length. The slurry of activated silica gel (column grade 100-200 mesh) prepared using petroleum ether was poured into the column and allowed to settle down, supervision was taken to keep away from any air space or bubble during packing. The silica gel was packed up to three-fourth of the column length and the solvent level was maintained 5 cm above the silica layer to avoid cracking and air entrapment.

Crude solid (50 g) was dissolved in a small quantity of suitable solvent to form a clear solution followed by the addition of 10 g actuated silica gel and mixed thoroughly. The solvent was then dried off completely and the sample adsorbed silica gel was uniformly placed on uppermost of the column, the protection was taken that the solvent level is always maintained 5 cm above the layer of silica gel. After stabilization, a filter paper disc was carefully fixed on uppermost of the silica gel. The methanolic extract of *Arisaema tortuosum* was levied to column chromatography and the fractions were eluted with the gradient polarity of solvent namely petroleum ether, chloroform, ethyl acetate and methanol. A total of 188 fractions were collected. Fractions were collected as 25 mL portion and monitored by TLC. Fractions (26-29) eluted from chloroform:ethyl acetate (1:1), fractions (30-37) eluted from chloroform:ethyl acetate (1:2), fractions (52-72) eluted from ethyl acetate: methanol (3:1), fractions (91-114) eluted from ethyl acetate: methanol (2:3), fractions (128-143) eluted from ethyl acetate: methanol (1:4) and fractions (144-160) eluted from ethyl acetate: methanol (1:9) were found to be homogeneous. All the fractions were combined and concentrated to one-fourth of its volumes. It was then kept in the refrigerator overnight. Six different homogenous fractions were collected, dried and named as FAT-1, FAT-2, FAT-3, FAT-4, FAT-5 and FAT-6 (fractions of *A. tortuosum*), respectively. The yield of the fractions FAT1 and FAT2 was too little, while the yield of fraction FAT3, FAT4, FAT5 and FAT6 was 45, 56, 48 and 50 mg, respectively. Four fractions were additionally analyzed for phytochemical screening to determine the nature of the isolated fractions. The phytochemical investigations of *Arisaema tortuosum* tuber revealed the presence of phytoconstituents. Fraction FAT-6 exhibits the presence of alkaloids, saponins, carbohydrates, glycosides, tannins and phenolic compounds and flavonoids. Fractions FAT-3 and FAT-5 indicate the presence of flavonoids, while fraction FAT-4 revealed the presence of alkaloids, glycosides, *etc*. (Table-1).

TABLE-1
PHYTOCHEMICAL SCREENING OF FRACTIONS

Phytoconstituents	FAT-3	FAT-6	FAT-4	FAT-5
Alkaloids	–	++	++	–
Glycosides	–	++	++	–
Carbohydrates	–	++	–	–
Tannins & phenolic compounds	–	++	–	–
Flavonoids	++	++	–	++
Steroids	–	–		–
Proteins and amino acids	–	–	–	–
Fixed oils and fat	–		–	–
Saponins	–	++	–	–

Anti-inflammatory assay: The carrageenan-induced edema model [20-22] was performed to assess the anti-inflammatory activity of fractions procured from *Arisaema tortuosum*.

Albino Wistar rats of either sex weighing between (150-200 g) were grouped by taking six animals in each group. The groups were as follows:

Group-I: Treated with distilled water (control group)

Group-II: Treated with standard drug Indomethacin at 10 mg/kg body weight

Group III: Treated with FAT-3 at 25 mg/kg BW
Group IV: Treated with FAT-4at 25 mg/kg BW
Group V: Treated with FAT-5 at 25 mg/kg BW

The mean paw volume was calculated and compared with control and standard groups. Depletion in the paw volume in separate fractions pretreated groups contrasted with the control animals were contemplated as an anti-inflammatory response. The percentage of paw edema inhibition was estimated by using the following formula.

$$\text{Inhibition } (\%) = \frac{V_c - V_t}{V_c} \times 100$$

where V_t = an increase in paw volume in rats treated with test compounds. V_c = an increase in paw volume in control.

Characterization: NMR spectral studies were carried out for the purified compounds PC-1 and PC-2 using Brucker NMR 600 MHz spectrophotometer using DMSO used as a solvent and TMS as an internal standard. Fourier transform infrared spectra were obtained by Thermo Scientific Nicolet 6700 system with 16 scans per sample at the range of 4000-400 cm^{-1}. The liquid chromatography combined with the mass spectrum (LC/MS) of compounds PC-1 and PC-2 were recorded on HRMS instrument at 70 eV by direct inlet method.

Statistical analysis: All values are given as mean ± SEM. The distinction between means was evaluated by one-way analysis of variance (ANOVA), accompanied by Dunnett's test; $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

In present study, the methanolic extract of *Arisaema tortuosum* was subjected to column chromatography. The fractions were eluted with the inclined polarity of solvents specifically petroleum ether, chloroform, ethyl acetate and methanol. Administration of fraction FAT3, FAT4 and FAT5 (25 mg/kg) revealed a significant ($p < 0.05$) reduction in edema compared to control treated group. Indomethacin (10 mg/kg) produced a significant ($p < 0.05$) decrease in edema at the 2 h compared to control treated group. The decreasing order of edema in the rats for fractions was FAT5 > FAT3; FAT-4, which did not show any significant activity (Table-2). Column fractions FAT-3 with ethyl acetate:methanol (3:1) in the mobile phase solvent ratio of ethyl acetate-methanol-water 15:3:2 (%, v/v) showed an R_f value of 0.40. The fraction FAT-3 was named as PC-1 (m.p. 255 ºC; m.f.: $C_{28}H_{34}O_{15}$). Column fractions FAT-5 with methanol: ethyl acetate (4:1) in The TLC mobile phase solvent ratio of ethyl acetate:formic acid:acetic acid:water (10:1:1:2.5) (%, v/v) showed R_f value of 0.93. The fraction FAT-5 was named as PC-2 (pale yellow amorphous powder, m.p. 232 ºC; m.f.: $C_{15}H_{10}O_5$).

Spectral data (PC-1): ^{1}H NMR (DMSO, 600 MHz): 4.521 (1H, br, s), 5.414 (1H, dd), 3.126 (1H, dd), 3.120 (1H, dd), 3.78 (3H, m), 12.024 (s, 5H, ex, 5-OH) 6.118 (1H, d), 1.085 (3H, d), 6.127 (1H, s), 6.939 (1H, s). ^{13}C NMR (DMSO, 150 MHz): 78.42 (C-2), 42.32 (C-3), 197.09 (C-4), 163.04 (C-5), 96.41 (C-6), 165.17 (C-7), 95.62 (C-8), 162.58 (C-9), 103.35 (C-10), 130.99 (C-1′), 114.18 (C-2′), 146.48 (C-3′), 148.00 (C-4′), 112.11 (C-5′), 118.01 (C-6′), 99.46 (C-1″), 72.10 (C-2″), 75.54 (C-3″), 69.62 (C-4″), 76.30 (C-5″), 66.12 (C-6″), 100.64 (C-1‴), 70.73 (C-2‴), 70.75 (C-3‴), 73.02 (C-4‴), 68.36 (C-5‴), 17.89 (C-6‴), 55.71 (-OCH_3). IR (KBr, ν_{max}, cm^{-1}): 3543.41 (O-H *str.*), 3082.04 (alkene), 2938.38 (alkane), 2918.68 (alkane –OCH_3), 1647.04 (C=O *str.*), 1276.48 (arom. C-O *str.*). EI-mass: $M^{+\bullet}$ at *m/z* of 633.21 (100%-relative intensity).

Based on the results FT-IR, MS and NMR spectrum, an isolated compound has been characterized as (2*S*)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-({[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyl-oxan-2-yl]oxy}methyl)oxan-2-yl]oxy}-3,4-dihydro-2*H*-1-benzopyran-4-one which agreed with the reported literature as hesperidin (Fig. 1). Hesperidin is a disaccharide imitative which comprised of hesperetin exchanged by a 6-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl moiety at position 7 *via* a glycosidic linkage. It is characterized as a mutagen and a member of 3′-hydroxyflavanones, a dihydroxyflavanone, a monomethoxyflavanone, a flavanone glycoside, a member of 4′-methoxyflavanones and a rutinoside [23].

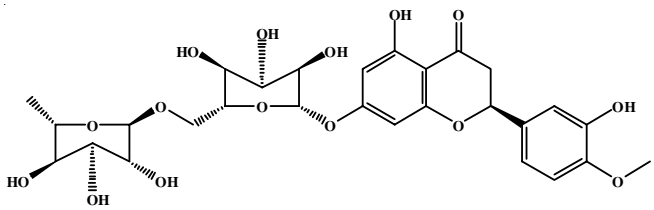

Fig. 1. Structure of PC-1 (hesperidin)

Spectral data (PC-2): ^{1}H NMR (DMSO, 600 MHz): 6.963 (1H, s), 12.660 (s, 3-OH), 8.821 (1H, s), 7.064 (1H, s), 8.059 (2H, d), 7.583-7.559 (3H, m). ^{13}C NMR (DMSO, 150 MHz): 162.92 (C-2), 104.50 (C-3), 182.15 (C-4), 146.98 (C-5), 153.65 (C-6), 149.85 (C-7), 94.03 (C-8), 130.98 (C-9), 104.50 (C-10), 131.85 (C-1′), 126.32 (C-2′), 129.34 (C-3′), 129.34 (C-4′), 131.85 (C-5′), 129.14 (C-6′), 126.32 (-OCH_3). IR (KBr, ν_{max}, cm^{-1}): 3091 (C–H *str.* (aryl)), 3400 (O–H *str.*), 1755 (C=O *str.*)

TABLE-2
ANTIINFLAMMATORY ACTIVITY OF SELECTED FRACTIONS

Group	Increase in paw volume (mm)			Inhibition (%)		
	1 h	2 h	3 h	1 h	2 h	3 h
Control	0.45 ± 0.01	0.58 ± 0.01	0.72 ± 0.02	–	–	–
FAT-3 (25 mg/kg BW)	0.28 ± 0.01*	0.43 ± 0.06*	0.34 ± 0.01*	37.26	26.10	52.27
FAT-4 (25 mg/kg BW)	0.44 ± 0.02	0.55 ± 0.01	0.67 ± 0.02	1.84	5.84	6.68
FAT-5 (25 mg /Kg BW)	0.25 ± 0.68*	0.34 ± 0.21*	0.28 ± 0.01*	46.12	34.95	60.80
Indomethacin (10 mg/Kg BW)	0.21 ± 0.01*	0.32 ± 0.006*	0.23 ± 0.02*	51.66	44.07	67.25

Values are expressed as mean ± SEM, n = 6 in each group. *$p < 0.05$ compared to the control group.

and 1550 (arom. C-C *str.*), 1490 (O-H bend.). EI-mass: $M^{+\bullet}$ at *m/z* of 271.07 (100% - relative intensity) and a $[M+H]^+$ peak at *m/z* of 272.08.

Based on the results FT-IR, MS and NMR spectrum, an isolated compound has been characterized 5,6,7-trihydroxy-2-phenyl-chromen-4-one, which agreed with the reported literature as baicalein (Fig. 2). Baicalein is a trihydroxyflavone having hydroxy groups at positions C-5, -6 and -7. It has a role as an antioxidant, a hormone antagonist, a prostaglandin antagonist, an arachidonate 12-lipoxygenase inhibitor, an arachidonate 15-lipoxygenase inhibitor, a radical scavenger, a prolyl oligopeptidase inhibitor, an anti-inflammatory agent and a plant metabolite [24].

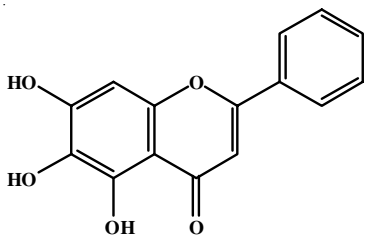

Fig. 2. Structure of PC-2 (baicalein)

Conclusion

In this work, *Arisaema tortuosum* was recognized as a fascinating source for anti-inflammatory interest. Subsequent bioactivity-guided isolation, hesperidin (PC-1) and baicalein (PC-1) were isolated and identified as the bioactive flavonoids from the methanolic extracts of *Arisaema tortuosum* tuber. The isolated PC-1 and PC-2 also showed a significant anti-inflammatory activity in carrageenan-induced rat paw edema method. However, PC-2 was found to be a more potent PC-1 in respect to the anti-inflammatory activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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