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Research Article

Isolation of Phytoconstituents and Evaluation of Anti-inflammatory Activity of the Leaves of *Abelmoschus manihot*

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ABSTRACT: The present study was carried out to isolate the phytoconstituents and evaluate the anti-inflammatory activity of the petroleum ether and methanol extract of *Abelmoschus manihot* (Malvaceae) **leaves** using paw edema model. The air-dried, powdered laeves (1 Kg) were extracted over Soxhlet with petroleum ether and methanol. Two steryl alcohol, named 1-dodecanol (**1**) and 1-tridecanol (**2**) from petroleum ether exctract, together with a known acid ester, 1-tridecanoic acid methyl ester (**3**) from methanol extract, were isolated from *Abelmoschus manihot*. Their structures were elucidated by spectroscopic methods. The crude dried petroleum ether (10 g) and methanol (25 g) extracts was prepared at doses of 100, 200 and 400 mg/kg, and evaluated for anti-inflammatory using the carrageenan and histamine-induced paw edema test. The results obtained indicate that the extracts possessed significant ($p < 0.01$) anti-inflammatory activity, which was found to be dose-dependent. This study showed that the petroleum ether and methanol extracts of *Abelmoschus manihot* woody stems possess potential pharmacological active constituents responsible for inhibition of the inflammation effect.

KEYWORDS: *Abelmoschus manihot,* Steryl alcohol, Acid methyl ester, Anti-inflammatory activity.

INTRODUCTION

Research studies leading to extraction, isolation and biological study of plant constituents have now formed **a** major field of study. *Abelmoschus manihot,* Malvaceae[1] is a large annual erect hairy plant, 1.2-1.8 m. high. It is **native** to China and **has been**, introduced into India, near Calcutta and in coastal areas of Maharashtra. The plants mucilage contains polysaccharides and proteins.[2] The flower contain quercetin-3-robinoside, quercetin-3'-glucoside, hyperin, myrecetin and anthocyanins. The saturated acids and liquid acids such as linoleic and oleic acids were isolated from the seed fat and unsaponifiable matters. The larvicidal activity of roots of *A. manihot* was evaluated against the larvae of mosquitoes in the genera Anapheles and Culex.[3] **Different** chromatographic methods have been developed **for the separation of** the flavones present in **this** plant.[4,5] The flavones present in the plant showed preventive effect **towards** injury.[6,7] The ethanol extract of flower was screened for antiviral activity, and it was observed that the hyperoside shown significant anti HBV activity.[8] The leaves were tested on bone loss in ovarectomised rats and it was observed that it was able to prevent the ovariectomy – induced femoral osteopenia.[9] The modulatory effect of **total flavone** of *Abelmoschus manihot* (TFA) on NMDA-activated current (I_{NMDA}) was investigated in cultured rat hippocampal neurons using the whole-cell patch-clamp technique. TFA rapidly and reversibly inhibited the I_{NMDA} in a concentration-dependent manner**.**[10] **However**, these studies **not conclusively identify** and **characterize** the bioactive compounds in this plant. The purpose of this study is **therefore,** to identify and characterize the bioactive principles from the leaves of *A. manihot*. **This** plant is very useful in the treatment of abdominal pain, skin diseases, antidiarrhoeal and antihaemorrha.[11] This study, therefore, intends to investigate the anti-inflammatory activity of the leaves of *A. manihot* by carrageenan induced and histamine induced rat paw edema model.

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MATERIALS AND METHDOS

General experimental procedures

UV spectra were measured with a Schimadzu UV-Visible spectrophotometer 1601. IR spectra were recorded on a

Shimadzu FTIR 8400 spectrophotometer. NMR spectra were obtained on a Varian Mercury-Plus 300, 400 or 500 Mhz spectrometer. The ^{1}HNMR and ^{13}CNMR spectra were recorded using $CDC1_3$, as solvent with Tetramethylsilane (TMS) as an internal standard. The HR-ESI-TOF–MS data were obtained at high resolution on a mass spectrometer (Perkin Elmer Autosystem XL with Turbomass). Column chromatography (CC) was conducted on silica gel (60-120 mesh) (Rankem Chemical, New Delhi), and ODS silica gel (Nacalai Tesque, Kyoto, Japan). TLC was performed on precoated silica gel 60 GF254 (0.25 mm thick, Loba chemie, New Delhi) and RP18 F254s plates (0.25 mm thick, Merck). HPLC was carried out using Agilent liquid chromatograph comprising G 1311A pump equipped with a G1315 diode array detector, UV detector and a Rheodyne injector with 20 µL loop. EZChrome Elite was used as a data processer. A Qualisil BDS C-18 column (20 × 250 mm, 5-µm) was used for chromatographic separation under suitable conditions.

Plant material

The leaves of *Abelmoschus manihot* Linn were collected from the Toranmal Hills of Maharashtra in August 2009 and have been identified by Prof. D. A. Patil, Dhule, Maharashtra, India, Department of Botany. A voucher specimen (Ref. No. PSJ/1235) has been deposited in the R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India.

Extraction and isolation

The leaves of *A. manihot* (1 kg) were powdered and extracted with petroleum ether followed by methanol four times by Soxhlet extraction. After evaporation of the solvents, 10 g and 25 g of petroleum ether and methanol extract was obtained respectively. The petroleum ether extract then saponified with alcoholic KOH, to remove fatty material, yielding 5 g of unsaponifiable matter. A small quantity of unsaponifiable matter was dissolved in chloroform and this solution was spotted on TLC plates. The TLC plates were run by petroleum ether: ethyl acetate (7:3 v/v) solvent system and were viewed individually under UV light and also with the ethanolic-H_2SO_4, reagent. Through several pilot experiments, it was found that the compounds of unsaponifiable matter were separated by the solvent system of petroleum ether: ethyl acetate (7:3 v/v)]. The unsaponifiable fraction, 5 g, was subjected to column chromatography on a silica gel (60-120 mesh) with gradient elution using petroleum ether: ethyl acetate [(9:1-8:2-7:3 v/v) yield compound 1 (17 mg), 2 (26 mg).

A small quantity of methanol extract was dissolved in methanol and this solution was spotted on TLC plates. Then the TLC plates were run by specific solvent system and were viewed individually under UV light and also with the Mg turnings and Hydrchloric acid, reagent. Through several pilot experiments, it was found that the compounds of methanol extract were separated by the solvent system of chloroform: methanol (1:1 v/v). The methanol extract, 5 g, was subjected to column chromatography on a silica gel (60-120 mesh) with gradient elution using chloroform: methanol [(6:1-4:1-2:1-1:1 v/v) to yield compound 3(35 mg).

1-Doceanol (1)

1-dodecanol was obtained as Orange liquid from petroleum ether: ethyl acetate (7:3), 17 mg, bp 259 °C. UVλ_{max} ($CHCl_3$):273 nm. IRυmax: 3419, 2926, 2854, 1465, 1215, 1053, 759, 669 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500MHz): δ3.64 (s, 1H, OH), δ 1.54 (t, 2H, CH_2), δ 0.88 (t, 3H, CH_3), δ 1.01-1.41 (m, 20H, CH_2). ^{13}C NMR ($CDCl_3$, 100MHz): spectral data are presented in Table 1. HR-ESI-TOF-MS MS: m/z 125(7), 140(8), 111(25), 97(55), 83(82), 57(82), 69(86), 55(100) (calculated for $C_{12}H_{26}O$, 186),

1-Tridecanol (2)

1-tridecanol was obtained as White, colorless solid from petroleum ether: ethyl acetate (7:3), 26 mg, mp 272 °C. UV λ_{max} ($CHCl_3$):285 nm. IR υmax: 3385, 2953, 2841, 1456, 1222, 1016, 771, 660 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500MHz): δ3.63 (s, 1H, OH), δ 1.56 (t, 2H, CH_2), δ 0.90 (t, 3H, CH_3), δ 1.10-1.41 (m, 22H, CH_2). ^{13}C NMR ($CDCl_3$, 100MHz): spectral data are presented in Table 1. HR-ESI-TOF-MS: m/z 154(4), 140(6), 125(8), 111(17), 97(41), 83(62), 55(100) (**calculated** for $C_{13}H_{28}O$, 200).

1-Tridecanoic acid methyl ester (3)

1-tridecanoiac acid methyl ester was obtained as white, colorless solid from chloroform: methanol(1:1), 35 mg, mp 42 °C. UVλ_{max} ($CHCl_3$):231 nm. IR υmax: 3020, 2928, 1732, 1465, 1215, 1045, 765 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500MHz): δ 3.62 (t, 1H, -OCH3), δ 1.52 (t, 2H, CH_2), δ 0.80 (t, 2H, -CH2), δ 1.20-1.40 (m, 20H, CH_2). ^{13}C NMR ($CDCl_3$, 100MHz): spectral data are presented in Table 1. HR-ESI-TOF-MS: m/z 185(2), 129(8), 143(9), 69(17), 74(100) (calculated for $C_{14}H_{28}O_2$, 228).

Pharmacological studies

Test animals

Wistar rats (150-200 g; 8-11 weeks old), and Swiss albino mice (25-30 g; 7-10 weeks old) were, obtained from RC Patel Institute of Pharmaceutical Education and Research, Shirpur and Government Veternary College, Mahu, MP (India). The animals were housed in Animal house of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India in polycarbonate cages, in a room maintained, under controlled room temperature 22 ± 2 °C, relative humidity 60-70% and provided with food and water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India. The animals were

deprived of food for 24 h before experimentation but allowed free access to water throughout the study. All studies were carried out by using six animals per group to monitor anti-inflammatory activity.

Acute toxicity test

Acute toxicity tests were performed according to OECD-2006 guidelines. Animals were weighed and marked, a single high dose, 2000 mg/kg of petroleum ether extract and methanol extract as recommended by the OECD guidelines was administered to the first animal. After a single administration, the signs of toxicity and behavior were observed each hour up to the 24 hour. If this animal was dying, then **a** lesser dose was administered to the next animal tested. The same procedure was followed for subsequent animals. If the animal survived, then the same dose was given to remaining animals. All the animals were observed for the signs of toxicity and mortality for up to the 14 days.

Additional observations like changes in skin, eyes and mucous membranes, and also respiratory circulatory, autonomic and Central Nervous system and behavior pattern were also recorded. Attention was also given to observed precipitation of tremors and convulsions.

Carrageenan-induced paw edema

Wistar Rats of either sex (150-200 g) were divided into eight groups containing six animals in each. The rats were fasted for 24 h prior to induction of edema however water was available *ad libitum*. Rats were deprived of water only during the experiment to ensure uniform hydration and minimize variability in edematous response. Inflammation of hind paw was induced by injecting 0.1 ml of 1% w/v carrageenan in normal saline into the subplantar region of right hind paw.[12] The negative control group received Saline: CMC (0.5%) solution[13] and the positive control group received Diclofenac sodium (10 mg/kg) p.o.[14] Three groups received petroleum ether extract orally at the doses 100, 200 and 400 mg/kg, respectively. The remaining three groups orally received methanol extract at doses 100, 200 and 400 mg/kg, respectively. All the drug treatments were given 1 hr before the carrageenan injection; edema was expressed as the increase in paw volume due to carrageenan injection. The paw volume was measured with a digital plethysmometer (Ugo Basile, 7140) before and 1, 2, 3, 4, 5 and 6 h after carrageenan injection.[15] The extracts and the reference drug were dissolved in 0.5% carboxy methyl cellulose solution just before use.

Histamine induced paw

Wistar rats of either sex weighing 150-200 g were divided into eight groups containing six animals each. The rats were fasted for 24 h prior to induction of edema however water was available *ad libitum*. Inflammation of hind paw was induced by injecting 0.1 ml of histamine (1 mg/ml) in normal saline into the subplantar region of right hind paw.[16] The negative control group received CMC (0.5%) solution[13] and the positive control group Diclofenac sodium (10 mg/kg) p.o.[14] Percentage rise in paw volume was determined by the formula stated below.

$$\% \text{ Rise} = \frac{Vt - Vc}{Vc} \times 100$$

Where, Vt = Paw volume post carrageenan injection t
Vc = Paw volume before carrageenan injection o

Data Statistical analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using graph pad and the results obtained in the study were compared with the vehicle control group. *P* values <0.05 were considered statistically significant.

RESULTS

Isolation and characterization of phytoconstituents

Compound 1, an orange liquid gave a molecular formula $C_{12}H_{26}O$ by HR-ESI-TOF-MS at m/z 186. The UV spectrum exhibited absorption maxima at 273 nm. Its IR spectrum disclosed absorption assignable to a very intensely broad band at 3419 cm^{-1} and intense band at 1215 and 669 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. The stretching and bending vibrations of methyl part were noticed by the intense band 2926 cm^{-1} and medium intensity band at 1465 cm^{-1}. The vibration of the methylene part was shown by the band at 2854 cm^{-1}. The moderately intense band at 759 cm^{-1} was attributed to the rocking movement of methylene part. The corresponding C-C vibration was shown as weak intense band at 1053 cm^{-1}. The ^{1}H NMR spectrum of compound 1 exhibited the following proton signals: singlet at δ 3.64 for hydroxyl group. The methylene group attached to hydroxyl group gives triplet at δ 1.54. The methyl group gives triplet at δ 0.88. The multiplet was observed at δ 1.01-1.41, by the remaining twenty hydrogen atoms. The ^{13}C-NMR spectra revealed twelve aliphatic carbon atoms (Table 1). The ^{1}H NMR ^{13}C-NMR spectra of compound 1 was similar to those of a known compound 1-dodecanol.[17] Thus, the structure of compound 1 was assigned as shown in Figure 1.

Compound 2, a colorless white solid gave a molecular formula of $C_{13}H_{28}O$ by HR-ESI-TOF-MS at m/z 200. The UV spectrum exhibited absorption maxima at 285 nm. Its IR spectrum disclosed absorption assignable to a very intensely broad band at 3385 cm^{-1} and moderately intense band at 1222 and 660 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. The stretching and bending vibrations of methyl part were noticed by the

Table 1: ^{13}C NMR spectral data (δ) of compound 1-3 siolated from leaves of *Abelmoshus manihot*

Position	1	2	3
1	68.87	68.87	51.4
2	40.04	44.08	34.1
3	32.56	40.04	31.7
4	30.27	38.87	29.6
5	26.43	32.56	29.4
6	24.33	30.27	29.2
7	23.33	26.43	25.2
8	23.31	25.2	24.11
9	23.11	24.11	23.33
10	20.33	23.33	23.11
11	14.85	23.11	18.5
12	14.75	14.85	18.2
13		14.75	14.85
14		14.75	

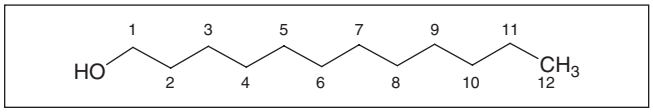

Figure 1: Chemical structure of 1- Dodecanol (1)

intense band 2953 cm^{-1} and medium intensity band at 1456 cm^{-1}. The vibration of the methylene part was shown by the band at 2841 cm^{-1}. The moderately intense band at 771 cm^{-1} was attributed to the rocking movement of methylene part. The corresponding C-C vibration was shown as weak intense band at 1016 cm^{-1}. The ^{1}H NMR spectrum of compound 2 exhibited the following proton signals: singlet at δ 3.63 for hydroxyl group. The methylene group attached to hydroxyl group gives triplet at δ 1.56. The methyl group gives triplet at δ 0.90. The multiplet was observed at δ 1.10-1.41, by the remaining twenty two hydrogen atoms. The ^{13}C-NMR spectra revealed thirteen aliphatic carbon atoms(Table 1). The ^{1}H NMR ^{13}C-NMR spectra of compound 2 were similar to those of a known compound 1-tridecanol.[17] Thus, the structure of compound 2 was assigned as shown in Figure 2.

Compound 3**,** a colorless solid gave a molecular formula of C_{14} H_{28} O_2 by HR-ESI-TOF-MS at m/z 228. The UV spectrum exhibited absorption maxima at 231 nm. Its IR spectrum disclosed absorption assignable to a very intensely sharp band at 1732 cm^{-1} was observed for the vibrations of carbonyl group. The stretching and bending vibrations of methyl part were noticed by the intense band 3020 cm^{-1} and medium intensity band at 1465 cm^{-1}. The vibration of the methylene part was shown by the band at 2928 cm^{-1}. The moderately intense band at 1215 was observed for the C-O-C bond. The moderately intense band at 765 cm^{-1} was attributed to the rocking movement of methylene part. The corresponding C-C vibration was shown as weak intense band at 1045 cm^{-1}. The ^{1}H NMR spectrum of compound 3 exhibited the

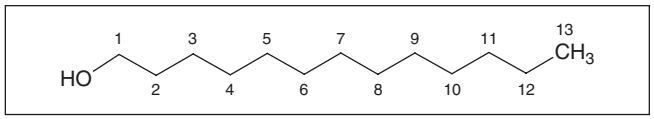

Figure 2: Chemical structure of 1-Tridecanol (2)

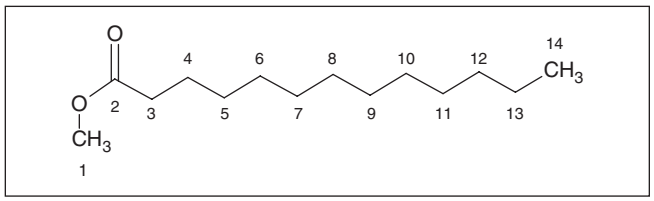

Figure 3: Chemical structure of 1-Tridecanoic acid methyl ester (3)

following proton signals: triplet at δ 3.62 was observed for OCH_3 group. The methylene group attached to carbonyl group gives triplet at δ 1.52. The methyl group gives triplet at δ 0.80. The multiplet was observed at δ 1.20-1.40, by the remaining twenty hydrogen atoms. The ^{13}C-NMR spectra revealed fourteen aliphatic carbon atoms(Table 1). The ^{1}H NMR ^{13}C-NMR spectra of compound 3 were similar to those of a known compound 1-tridecanoic acid methyl ester.[18] Thus, the structure of compound 3 was assigned as shown in Figure 3.

Acute toxicity

Abelmoschus manihot woody stem extracts did not produce any mortality even at the dose of 2000 mg/kg, p.o. *A. manihot* was thus found to be non-toxic, the results showed no clinical signs and mortality of the animal therefore an LD_{50} > 2000 mg/kg body weight may be assumed. On the basis of above results, three doses (100, 200, 400 mg/kg, p.o.) of *A. manihot* was selected for further pharmacological studies.

Carrageenan-induced rat paw edema

The petroleum ether and methanol extracts at the doses of 100, 200 and 400 mg/kg p. o. showed very good results and caused a significant inhibition in the percent rise of carrageenan induced rat paw edema. The 400 mg/kg dose of petroleum ether extract showed percent rise inhibition of 71.7 ± 6.26 and of methanol extracts showed 86.8 ± 7.54, at 3rd hour as highly significant results (Table 2). The maximal inhibition in the percent rise of edema volume was achieved at a dose 400 mg/kg (P <0.01) of petroleum ether and methanol extracts, when compared to standard drug diclofenac sodium (10 mg/kg).

Histamine induced rat paw edema

The petroleum ether and methanol extract at the doses of 100, 200 and 400 mg/kg p. o. showed very good results and caused significant inhibition in the percent rise of Histamine induced rat paw edema. The 100 mg/kg dose of petroleum ether extract showed percent rise inhibition of 41.2 ± 2.14

Table 2: The anti-inflammatory activity of petroleum ether and methanol extract of *Abelmoschus manihot* using the carrageenan-induced paw edema test

Time in hours	Control	Petroleum ether extract 100 mg /Kg	Petroleum ether extract 200 mg /Kg	Petroleum ether extract 400 mg /Kg	Methanol extract 100 mg /Kg	Methanol extract 200 mg /Kg	Methanol extract 400 mg /Kg	STD 10 mg/Kg
1	51 ± 3.97	38.2 ± 1.92	33.2 ± 5.49**	32.3 ± 4.17**	38.6 ± 6.73*	41.4 ± 3.79*	27.6 ± 2.13**	18.1 ± 1.01**
2	69 ± 4.44	56.6 ± 2.00	42.5 ± 1.46*	44.9 ± 5.89**	51.3 ± 10.4	48.1 ± 2.74	41.7 ± 4.19**	20.2 ± 0.49**
3	91.4 ± 1.98	66.1 ± 3.78	59.7 ± 4.22**	71.7 ± 6.26**	64.2 ± 8.06	84.5 ± 4.95	86.8 ± 7.54*	46.5 ± 1.82**
4	81.9 ± 4.75	55.5 ± 2.96**	46.3 ± 2.49**	43.6 ± 6.75**	60.6 ± 6.23	48.1 ± 4.2*	49.1 ± 6.9	38.1 ± 3.6**
5	83.1 ± 3.78	52.8 ± 2.70	50.8 ± 3.15**	45.6 ± 5.47**	75.9 ± 5.03	61.4 ± 4.87**	67.7 ± 5.55	35.3 ± 4.83**
6	84.5 ± 4.22	43.1 ± 4.40**	40.9 ± 6.3**	27.9 ± 7.05**	69.6 ± 5.24	59.5 ± 1.42**	62.9 ± 6.76*	30.7 ± 4.05**

Values represent mean ± SEM, n = 6
One way ANOVA followed by Dunnett's multiple comparison test
*p < 0.05, **p < 0.01 compare with control group

Table 3: The anti-inflammatory activity of Petroleum ether and methanol extract of *Abelmoschus manihot* Assessed using the histamine-induced paw edema test

Time in hours	Control	Petroleum ether extract 100 mg /Kg	Petroleum ether extract 200 mg /Kg	Petroleum ether extract 400 mg /Kg	Methanol extract 100 mg /Kg	Methanol extract 200 mg /Kg	Methanol extract 400 mg /Kg	STD 10 mg/Kg (Diclofenac)
1	56.2 ± 3.02	41.2 ± 2.14*	26.0 ± 2.46**	20.8 ± 5.09**	35.0 ± 1.55**	40.1 ± 4.60*	38.4 ± 5.47**	14.7 ± 0.835**
2	72.6 ± 2.62	40.2 ± 2.76**	24.5 ± 0.893**	14.9 ± 2.40**	30.4 ± 1.14**	37.8 ± 4.76**	29.1 ± 6.37**	11.64 ± 1.18**
3	76.6 ± 4.32	37.5 ± 2.90**	20.9 ± 1.44**	12.3 ± 2.21**	26.5 ± 0.940**	28.4 ± 4.78**	24.1 ± 5.99**	10.13 ± 0.954**

Values represent mean ± SEM, n = 6
One way ANOVA followed by Dunnett's multiple comparison test
*p < 0.05, **p < 0.01 compare with control group

and 200 mg/kg of methanol extract showed 40.1 ± 4.60, at 1st hour as highly significant results (Table 3). The maximal inhibition in the percent rise of edema volume was achieved at a dose 400 mg/kg (P <0.01) of petroleum ether and methanol extracts, when compared to standard drug diclofenac sodium (10 mg/kg).

DISCUSSION

The present study indicates that *Abelmoschus manihot* has the pharmacological potential as an anti-inflammatory and agent when tested on various animal models. Although this study did not aim at isolation and identification of bioactive compounds, the phytochemical screening of petroleum ether and methanol extract demonstrated the presence of flavonoids, steroids, triterpenoids, which are suggested to act synergistically to exert the observed pharmacological activity,[19] the presence of steroids and flavonoids in petroleum ether and methanol extract could possibly lead to the observed activities. The anti-inflammatory activity of petroleum ether and methanol extract could also be linked to the ability of the extract to influence the peripheral and central COX activity or prostaglandin synthesis.[20] This fact is supported by claims that the carrageenan-induced inflammation is a COX-dependent response and is more effectively controlled with arachidonate cyclo-oxygenase but not arachidonate lipo-oxygenase inhibitors.[21] The ability of petroleum ether and methanol extract to inhibit/reverse the effect of prostaglandins or COX has been explained in the above paragraph.

Interestingly, compounds like flavonoids[22] and steroids, triterpenes,[23] in part, have been shown to possess anti-inflammatory activity, and the claim made by Attaway and Zaborsky[24] that compounds with anti-inflammatory activity also possess antinociceptive activity seems to support our findings on the petroleum ether and methanol extracts pharmacological activity. To demonstrate whether petroleum ether and methanol extract is producing anti-inflammatory activity in this model by acting on histamine, further the effect of petroleum ether and methanol extract was studied on histamine induced inflammation.

Histamine induced rat paw inflammation is the model used to study the anti-inflammatory activity of various agents. Histamine is one of the important mediators of inflammation. Histamine increase vascular permeability and act with prostaglandins to induce edema.[25,26] These mediators are stored in the secretory granules and are released from mast cells during their activation. They are proposed to act through the specific receptors on nearby vasculature and to induce plasma extravasation.[27]

In the histamine induced rat paw inflammation model, petroleum ether and methanol extract and the reference

drug Diclofenac sodium significantly decreased the inflammation at the 1st hour after histamine injection. At the second hour, the petroleum ether and methanol extracts show a characteristic decrease in paw edema and again decrease at the third hour, followed by further slowly decreases. In the late phases of this model**,** the petroleum ether and methanol extracts and reference drug Diclofenac sodium showed anti-inflammatory activity. The present results support the ethno-medical application of *Abelmoschus manihot leaves* in the treatment of inflammation diseases. Further experimentation is needed in order to understand the precise mechanism of action in anti-inflammatory activities by the extracts.

CONCLUSION

The phytochemistry of leaves Abelmoschus manihot was studied and from these physical, chemical and spectral evidences the isolated phytoconstitunets were confirmed as 1-dodecanol, 1-tridecanol and 1-tridecanoic acid methyl ester. The pharmacological activity indicates that the petroleum ether and methanol extracts possess anti-inflammatory activity. Thus, the present study confirmed the folklore use of *A. manihot* fruit for the treatment of various ailments, and the plant's potential pharmacological activities merit further investigation.

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