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Phytochemical and pharmacological studies on plant waste materials

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ABSTRACT

The present study was designed to evaluate the phytochemical profile and the anti-inflammatory, analgesic and anti-convulsant activity of methanolic extracts of Allium sativum, L.(peel of the bulb), Arachis hypogaea, L. (fruit shell), Citrus limon, L. (rind of the fruit), Punica granatum, L.(rind of the fruit) and Vitis vinifera, L.(seed).

Keywords: *Allium sativum*, *Arachis hypogaea*, *Citrus limon*, *Punica granatum*, *Vitis vinifera*, phytochemical, anti-inflammatory activity, analgesic activity and anti-convulsant activity.

INTRODUCTION

Herbal medicines have been used since the dawn of civilization to maintain health and to treat diseases. World Health Organization (WHO) estimates that about three-quarters of the world's population currently use herbs and other forms of traditional medicine to treat diseases [1]. Natural products have been used extensively in various cultures around the world as traditional medicines. The use of higher plants and their extracts to treat infection is an age old practice in traditional medicine [2]. The use of herbs is the most ancient approach for healing the ailments [3]. Man's dependence on plants from his life began since human race [4]. Medicinal plants have been used for many centuries to treat various diseases, which contain biologically active substances in their extracts, which have an inhabiting action towards the microbes [5].

The biologically active phytochemicals are normally present in leaves, roots, barks, flowers, stems etc., but the plant parts such as rind of the fruit, seed and fruit shell etc., normally treated as a waste and only a few reports is recorded in the waste part. But in this planet, no part of the plant is completely worthless. Each one has some biologically active phytochemical. Keeping this in mind the present work is performed on the following plant waste materials.

The peel of bulb of *Allium sativum, L.* (Liliaceae), locally known as vellapundu in Tamil language, the fruit shell of *Arachis hypogaea, L.*(Papilionaceae), locally known as 'vaerkadalai' in Tamil language, the rind of fruit of *Citrus limon, L.*(Rutaceae), locally known as 'elimicham' in Tamil language, the rind of fruit of *Punica granatum, L.* (Lythraceae), locally known as 'madali' in Tamil language and the seeds of *Vitis vinifera, L.* (Vitiaceae), locally known as 'draskha' in Tamil language [6, 7].

Previously isolated constituents and reported activities

Pectin [8-10], Six phenylpropanoids [11] and antioxidant activity [11] has been reported for *Allium sativum, L.* (peel of the bulb)]. No report has been made for *Arachis hypogaea, L.* (fruit shell). C-glucosylflavone [12], Volatile compounds [13], New aldehydes and related alcohols [14] and Insecticidal activity [15] has been reported for *Citrus limon, L.* (rind of the fruit). Polyphenolic compounds [16], Wound healing activity [17], antioxidant and antibacterial activity [18], antiliperoxidant activity [19] has been reported for *Punica granatum, L.* (rind of the fruit) Oligomeric and Polymeric Proanthocyanidins [20], antibacterial and antioxidant activity [21] has been reported for *Vitis vinifera, L.* (seed):

MATERIALS AND METHODS

Plant material

The peel of bulb of *Allium sativum, L.* the fruit shell of *Arachis hypogaea, L.* the rind of fruit of *Citrus limon, L.* the rind of fruit of *Punica granatum, L.* and the seeds of *Vitis vinifera, L.* were collected from Pottalpudur market near Alwarkurichi in the month of January.

Preparation of cold extract

The plant waste materials were cut into small pieces and dried under shade to a constant weight. Then percolated in methanol for 5 days with occasional shaking. After 5 days the extract was filtered and evaporated. This extracts were subjected to preliminary phytochemical screening [22-24] for the presence of steroids, triterpenoids, reducing sugar, alkaloids, phenolic compounds, saponin, xanthoprotein, tannin, flavonoids and aromatic acids (Table 1).

Animals

Albino rats of wistar strain weighing 150-20 g and male albino wistar rats weighing 200-250 g were procured from the animal house. Permission from animal ethical committee has been obtained to carry out experimentation in Department of Pharmacology, K.M. College of Pharmacy, Madurai(*IAEC NO. KMCT/07/60/IAEC/0017*). They were housed in standard polypropylene cages and kept under controlled room temperature in a 12 hr light-dark cycle. The rats were given a standard laboratory diet and water *ad libitum*.

Anti-inflammatory activity

The anti-inflammatory activity of methanol extracts of plant waste material on carrageenan induced paw odema was determined [25, 26]. The animals were divided into seven groups consisting of four animals each. The control group received 0.5 ml of dimethyl sulfoxide (DMSO) orally, the standard group received Diclofenac sodium (50 mg/ kg) and the test groups received the methanol extract at the dose of 50 mg/ kg, i.p. (Intra Peritoneal). Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan

with normal saline, in the right hind paw of the rats. One hour after administration of the drug, the paw odema was measured plethysmometrically at 0, 30 min, 1 hr, 2 hr and 4 hr after the carrageenan injection (Table 2).

Analgesic activity

The analgesic activity of the extracts was screened by employing tail-flick method[27, 28]. The animals were divided into seven groups of four animals each. The control group received 0.5 ml of dimethyl sulfoxide (DMSO) orally, the standard group received aspirin (200 mg/kg) and the test groups received the methanol extract at the dose of 50 mg/kg, i.p. Tail-flick response was evoked by placing rat tail over a wire heated electrically. The intensity of heat was adjusted so that the base line tail flick lateray averaged 3-4 set in all animals. Cut off period of 15 sec was observed to prevent the damage to the tail (Table 3).

Table 1 Preliminary phytochemical screening of methanolic extract of plant waste material

Plant	Steroids	Tri-terpenoids	Reducing Sugar	Alkaloids	Phenolic Compound	Saponin	Xantho protein	Tannin	Flavonoids	Aromatic acids
A. sativum,	-	-	+	-	-	+	-	+	+	+
A. hypogaea	-	-	+	-	-	-	-	+	-	-
C. limon	-	-	+	-	-	+	-	+	+	+
P. granatum	-	+	+	-	+	+	+	+	+	+
V. vinifera	-	+	+	-	+	+	+	+	+	+

+= Present; - =Absent

Table 2 Anti-inflammatory activity of methanolic extract of plant waste material

S. No	Treatment	Dose	Increase in paw volume in ml Mean ±SEM	% Inhibition of paw odema
1	Control	0.5 ml	4.12± 0.216	---------
2	Diclofenac sodium	50 mg/kg I.P	1.04± 0.126	74.75%
3	*A. Sativum*	50 mg/kg	3.48± 0.204	15.53%
4	*A. hypogaea*	50 mg/kg	3.28± 0.072	20.38%
5	*C. limon*	50 mg/kg	2.06± 0.128	50%
6	*P. granatum*	50 mg/kg	3.40± 0.168	17.47%
7	*V. vinifera*	50 mg/kg	3.52± 0.118	14.56%

Anti-Convulsant activity

The anti-convulsant activity of the extract was determined [29, 30]. Male albino wistar rats were divided into seven groups of four animals each. The control group received 0.5 ml of dimethyl sulfoxide (DMSO) orally, the standard group received phenytoin sodium (25 mg/kg) and the test groups received the methanol extract at the dose of 50 mg/kg, i.p. The evaluation was started 30 minutes after administration of test compounds. Pinna electrode with the intensity of 150 mA

current was used to deliver the stimuli. Inhibition of seizure relative to the control was calculated. The percentage of rats, which don't show hard limb extension, was calculated (Table 4).

Table 3 Analgesic activity of methanolic extract of plant waste material
(Values are significantly different from control at (P<0.05)

S. No	Treatment	Dose	Time in seconds				
			0 min	30 min	60 min	120 min	180 min
1	**Control**	0.5 ml	**2.4± 0.22**	**2.2± 0.23**	**2.0± 0.16**	**1.9± 0.28**	**2.5± 0.19**
2	Aspirin	200 mg/kg	2.4± 0.21	4.3± 0.16	6.2± 0.32	6.0± 0.31	6.4± 0.20
3	*Allium sativum*	50 mg/kg	2.6± 0.26	3.1± 0.18	3.4± 0.18	3.2± 0.16	3.0± 0.21
4	*Arachis hypogaea*	50 mg/kg	2.0± 0.19	2.8± 0.16	3.3± 0.11	3.6± 0.26	3.4± 0.32
5	*Citrus limon*	50 mg/kg	2.2± 0.13	3.6± 0.16	5.2± 0.09	5.4± 0.23	5.2± 0.08
6	*Punica granatum*	50 mg/kg	2.3± 0.14	3.8± 0.18	5.4± 0.11	5.0± 0.12	5.1± 0.26
7	*Vitis vinifera*	50 mg/kg	2.4± 0.18	3.0± 0.22	3.1± 0.29	2.8± 0.30	2.9± 0.32

Values were expressed as Mean ± SEM
Values were found out by using one-way ANOVA followed by Newman Keul's multiple range tests.

Table 4 Anti-Convulsant activity of methanolic extract of plant waste material

S. No	Treatment	Dose	Duration of extension phase in seconds	% Inhibition of extension phase
1	Control	0.5 ml	13.48± 0.36	---------
2	Phenytoin sodium	25 mg/kg	1.23± 0.48	90.87%
3	*Allium sativum*	50 mg/kg	5.68± 0.40	57.86%
4	*Arachis hypogaea*	50 mg/kg	9.4± 0.48	30.26%
5	*Citrus limon*	50 mg/kg	10.8± 0.56	19.88%
6	*Punica granatum*	50 mg/kg	6.56 ± 0.42	51.33%
7	*Vitis vinifera*	50 mg/kg	4.86 ± 0.16	63.94%

Values are expressed as Mean ± SEM

RESULTS AND DISCUSSION

Preliminary phytochemical screening is reported in table 1, anti-inflammatory activity, analgesic activity and anti-convulsant activity in tables 2, 3, and 4.In the phytochemical screening of methanolic extract of plant waste material compounds like reducing sugar, tannins are found to be present. These substances are chemotaxonamical markers. In *Allium sativum, L.*(peel of the bulb) and *Citrus limon, L.* (rind of the fruit) saponin, flavonoids and aromatic acids are found to be present .In *Punica granatum, L.*(rind of the fruit) and *Vitis vinifera, L. (seed)* , triterpenoids, phenolic compounds, saponin, xanthoprotein, flavonoids and aromatic acid are found to be present.The anti-inflammatory activity of methanolic extract *Allium sativum, L.*

(peel of the bulb), *Arachis hypogaea, L.* (fruit shell), *Citrus limon, L.* (rind of the fruit), *Punica granatum, L.* (rind of the fruit) and *Vitis vinifera, L.* (seed) reveals that the acute inflammation model of methanolic extract of *Citrus limon, L.* (rind of the fruit) and the standard drug produced significant inhibition of paw odema as compared to the control. All other extracts were found to be less effective than diclofenac sodium. The active principle responsible for the anti-inflammatory activity of *Citrus limon, L.* (rind of the fruit) may be the presence of reducing sugar, saponin, tannin, flavonoids and aromatic acid.The analgesic activity of methanolic extract of *Allium sativum, L.* (peel of the bulb), *Arachis hypogaea, L.* (fruit shell), *Citrus limon, L.* (rind of the fruit), *Punica granatum, L.*(rind of the fruit) and *Vitis vinifera, L.*(seed) reveals that *Citrus limon, L.*(rind of the fruit), *Punica granatum, L.*(rind of the fruit) and standard drug increased pain threshold ie., possess significant analgesic activity compared to control . The active principle responsible for the analgesic activity of *Citrus limon, L.* (rind of the fruit) and *Punica granatum, L.*(rind of the fruit) may be triterpenoids, reducing sugar, phenolic compounds, saponin, xanthoprotein, tannin, flavonoids and aromatic acids.

The anti-convulsant activity of methanolic extract of *Allium sativum, L.* (peel of the bulb), *Arachis hypogaea, L.* (fruit shell), *Citrus limon, L.* (rind of the fruit), *Punica granatum, L.*(rind of the fruit) and *Vitis vinifera, L.*(seed) reveals that *Allium sativum, L.* (peel of the bulb), *Punica granatum ,L.*(rind of the fruit)and *Vitis vinifera, L.*(seed) possess significant anti-convulsant activity. Among these *Vitis vinifera, L.* (seed) significantly abolish the duration of extensor phase when compare to control. The active principle responsible for the anti-convulsant activity of *Vitis vinifera, L.* (seed) may be triterpenoids, reducing sugar, phenolic compounds, saponin, xanthoprotein, tannin, flavonoids and aromatic acids.

CONCLUSION

The phytochemical and pharmacological studies on plant waste materials, in the anti-inflammatory activity analysis *Cirtus limon, L.* (rind of the fruit) showed significant anti-inflammatory activity and others are less active. In the analgesic activity analysis *Cirtus limon, L.* (rind of the fruit), *Punica granatum, L.* (rind of the fruit) showed significant analgesic activity and others are less active. In the anti-convulsant activity analysis *Allium sativum, L.* (peel of the bulb), *Punica granatum, L.* (rind of the fruit) and *Vitis vinifera, L.*(seed) possess significant anti-convulsant activity. Among these *Vitis vinifera, L.* (seed) high significant anti-convulsant activity. So in this planet, no part of the plant is completely worthless.

In future the efficiency may be improved if proper phytochemical and pharmacological studies are carried out effectively; the plant waste material may be used even in medicine to cater to the needs of ailing patients.

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