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SCREENING OF PHYTOCHEMICALS AND ANTIINFLAMMATORY ACTIVITY OF *Dolichandrone falcata* L

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ABSTRACT

A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. Anti-inflammatory drugs are presently available for the treatments of joint inflammation of various kinds and pathological state have undesirable side effects such as peptic ulcers. Therefore, plant remedies have become increasing popular and are often preferred to synthetically derived pharmaceuticals. Therefore, agents of natural origin with very little side effects are required as substitute chemical therapeutics. This present investigation was carried out to screen the Phytochemicals and *in vitro* anti-inflammatory activity of the leaves of *Dolichandrone falcata*.

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INTRODUCTION

World Health Organization estimate over 80% of the people in developing countries depend on traditional medicines for their primary health needs (Shankar and Majumdar, 1993). India is one of the largest producers of medicinal herbs and is rightly called the botanical garden of the world as it is sitting on a gold mine of well-recorded and traditionally well practiced knowledge of herbal medicine. About 17,000 species of Indian flora about 7500 species of higher plants are reported to possess medicinal value and in other countries it is projected about 7% and 13%. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India (Sunita Verma, 2016).

The search for new molecules, nowadays, has taken a slightly different route where the science of ethnobotany and ethnopharmacognosy are being used as guide to lead the chemistry towards different sources and classes of compounds (Gurib-Fakim, 2006). Plant derived natural products hold great promise for discovery and development of new pharmaceuticals (McChesney *et al*., 2007). The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutic for treatment of various inflammatory diseases. Though these drugs have potent activity,they have various and severe adverse effects. A number of natural products are used in the traditional medical systems in many countries.

Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. Therefore, agents of natural origin with very little side effects are required as substitute of chemical therapeutics. This present investigation was carried out to screen the Phytochemicals and *in vitro* anti-inflammatory activity of the leaves of *Dolichandrone falcata*.

MATERIALS AND METHODS

Plant materials:

The leaves of *Dolichandrone falcata* were collected in January 2017 from Sadaiyar kovil, Thajavur district, Tamil Nadu, India.

Preparation of alcoholic extract

The leaves of *Dolichandrone falcata* were first washed well and dust was removed from the leaves. The leaf was dried at room temperature and coarsely powdered. The powder was extracted with aqueous, methanol and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Quantitative analysis of phytochemicals

Determination of total phenols by spectrophotometric method. Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Saponin determine by the method of of Obadoni and Ochuko (2001). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013).

Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

***IN-VITRO* ANTI-INFLAMMATORY ACTIVITY**

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*.,(2012). *In vitro* anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*., (2012). Anti-inflammatory activity evaluated by Membrane stabilizing activity as described by Divya Singh *et al*., (2013)

Statistical analysis:

The results were presented as the mean ± SD. Data was statistically analyzed using students't' test.

RESULTS AND DISCUSSION

Anti-inflammatory drugs are presently available for the treatments of joint inflammation of various kinds and pathological state have undesirable side effects such as peptic ulcers. Therefore, plant remedies have become increasing popular and are often preferred to synthetically derived pharmaceuticals. Hence the present study deals with the screening of phytochemicals and anti-inflammatory activity of *Dolichandrone falcate.*

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Dolichandrone falcata* plant investigated and summarized in Table-1. The phytochemical screening aqueous extract of *Dolichandrone falcata* leaves showed that the presence of tannin, steroids, saponins, terepenoids, phenolics, glycosides, Flavonoids, triterpenoids, alkaloids. (Fig.2) Methanol extract of *Dolichandrone falcata* leaves showed that the presence of steroids, saponins, triterpenoids, phenolics, carbohydrate, anthroiquinone and glycosides, flavonoids, tannin, terepenoids, and protein. (Fig.3) Hydro-Methanolic extract of *Dolichandrone falcata* leaves showed that the presence of saponins, triterpenoids, phenolics, carbohydrate and glycosides, flavonoids, tannin and terepenoid., Significant amount of Flavonoids

(168mg/gm), Phenol (300mg/gm), Terpenoids (50mg.gm) and Saponin (33mg/gm).

Qualitative analysis of vitamins in *Dolichandrone falcata*

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004). Vitamin E, vitamin C, and beta carotene are antioxidants. Some studies suggest that the trio might help to strengthen the body's immune system and play a role in cancer prevention (Okwu, 2004). The best sources for vitamin C are citrus fruits, strawberries, melons, and stemy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times (Okwu, 2003). The vitamins of the *Dolichandrone falcata* investigated and summarized in Table-2. The vitamin analysis of *Dolichandrone falcata* stem showed that the presence of Vitamin C, E, D and A.

Identification of bioactive compounds in *Dolichandrone falcata* leaves extract by GC MS analysis

Twenty compounds were identified in *Dolichandrone falcata* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 3and Fig 4). The prevailing compounds Diethanolamine, 1,6,6-Trimethyl-9-Hydroxybicyclo, 2,4-Dinitrophenyl crotonate, 1,2-Dodecanediol and 2,4-Dinitrophenyl crotonate were found in this plant. The biological activity of the selected compounds were listed in Table 4. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results

***In vitro* anti-inflammatory activity**

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property *Dolichandrone falcata*. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant *et al*., 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium (Jagtap *et al.,* 2011). *Dolichandrone falcata* exhibited anti-inflammatory activities in dose dependent manner (Table 4-6).

Human Red Blood Cell (HRBC) method was selected for the in vitro evaluation of Anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Chippada and Meena, 2011). The hypotonicityinduced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Kumar *et al.,* 2012). The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium (Jagtap *et al.,* 2011). *Dolichandrone falcata* exhibited anti-inflammatory activities in dose dependent manner (Table 5-7).

Overall, the *Dolichandrone falcata* leaves are a rich source of phytochemicals and anti-inflammatory activity that can be important in inflammatory disease prevention including arthritis. In future isolation of this molecules responsible for the activity will be carried out which may be beneficial for the development of new anti-inflammatory agent.

Table: 1. Phytochemical screening of *Dolichandrone falcata*

S.No	Phytochemical analysis	Aqueous	100% Methanol	70% Methanol	Quantitative analysis (mg/gm)
1	Tannin	+	+	++	---
2	Phlobatannins	-	---	---	---
3	Saponin	+	+	+	33
4	Flavonoids	+	+	++	168
5	Steroids	-	+	---	---
6	Terpenoids	+	+	++	50
7	Tritrpenoids	-	+	+	---
8	Alkaloids	+	---	+	-
9	Carbohydrate	-	+	+	---
10	Protein	-	+	---	---
11	Anthroquinone	-	+	---	---
12	Polyphenol	+	++	+	300
13	Glycoside	+	+	++	---

(+) Presence; (-) Absence; (++) Higher concentrations

Table: 2. Qualitative analysis of vitamins in *Dolichandrone falcata*

S.No	Test	Qualitative Result
1.	Vitamins A	--
2.	Vitamins C	+
3.	Vitamins D	--
4.	Vitamins E	++

(+) Presence (-) Absence (++) High concentration

Table: 3. Identification of bioactive compounds in *Dolichandrone falcata* leaves extract by GC- MS analysis

Peak	R.Time	Area %	Height %	Molecular Formula	Name	Molecular Weight
1	15.552	3.35	6.66	$C_{20}H_{30}O_4$	Phthalic acid, heptyl pentyl ester	334
2	18.408	3.37	1.88	$C_{22}H_{17}NO_2$	2-(4'-Methylphenyl)-3-Benzoyl-4-P	327
3	18.729	4.35	6.06	$C_{23}H_{26}O_5$	4,4-Bis (Butoxycarbonyl) Benzoph	382
4	18.892	2.89	1.98	$C_{10}H_{13}NO_3$	Alanine, 3-(Benzyloxy)-, L-	195
5	19.743	8.77	17.77	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic Acid, Dibutyl	278
6	19.942	3.82	1.76	$C_3H_{10}OSi$	Silanol, Trimethyl-	90
7	20.856	8.41	9.50	$C_{12}H_{26}O_2$	1,2-Dodecanediol	202
8	21.121	8.05	5.03	$C_{13}H_{20}O_4Si$	Benzeneacetic acid, 4-methoxy-.alpha.-[(trimethylsilyl	268
9	21.283	9.75	5.48	$C_{12}H_{22}N_2O$	1,6,6-Trimethyl-9-Hydroxybicyclo	210

10	21.592	3.33	3.20	$C_{16}H_{24}O_4$	Methyl 1-(4-Methyl-3-Pentenyl)-2,4-Hexadien	280
11	21.997	6.02	3.29	$C_4H_{11}NO_2$	Diethanolamine	105
12	22.567	4.26	2.28	$C_{10}H_8N_2O_6$	2,4-Dinitrophenyl crotonate	252
13	22.847	5.52	6.61	$C_8H_{22}OSSi_2$	3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-tetramethyl	222
14	23.132	7.51	4.39	$C_5H_3F_7O_2$	Butanoic acid, heptafluoro-, methyl ester	228
16	26.020	2.31	3.30	$C_{11}H_{11}F_5N_2O$	p-Phenylenediamine, N,N-dimethyl-N'-pentafl	282
17	26.160	4.51	2.90	$C_4H_{14}OSi_2$	Disiloxane, 1,1,3,3-tetramethyl-	134
18	28.249	3.17	5.09	$C^{14}H^{24}O^3Si^2$	Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl	296
19	28.730	4.00	5.96	$C^{24}H^{38}O^4$	1,2-BenzenedicarboxYlic Acid, Diisooctyl	390
20	30.141	2.28	3.77	$C^6H^{18}O^3Si^3$	Cyclotrisiloxane, Hexamethyl-	222

Table :4. Biological activity of selected bioactive compounds in *Dolichandrone falcata* leaves extract by GC-MS analysis

Peak	R.Time	Molecular Formula	Name	Biological activity
1	21.99	$C_4H_{11}NO_2$	Diethanolamine	Insecticidal activity
2	21.28	$C_{12}H_{22}N_2O$	1,6,6-Trimethyl-9-Hydroxybicyclo	Nematocidal activity
4	22.56	$C_{10}H_8N_2O_6$	2,4-Dinitrophenyl crotonate	Antibacterial, Sedative, Fungicide
5	20.856	$C_{12}H_{26}O_2$	1,2-Dodecanediol	**Anti-inflammatory**, Antifungal activity
7	22.56	$C_{10}H_8N_2O_6$	2,4-Dinitrophenyl crotonate	Antibacterial activity

Table: 5. *In vitro* anti-inflammatory activity of *Dolichandrone falcata* (Egg albumin)

S.No	Doses (µg/ml)	Plant extract	Standard (Diclofenac sodium)
1	100	44.88 ± 0.83	21.37±1.98
2	200	50.01 ± 1.78	36.45±2.37
3	300	58.44 ± 0.83	55.94±3.47
4	400	74.92 ± 1.38	79.45±4.65
5	500	93.96 ± 0.54	93.45±6.84

Values are expressed as Mean ± SD for triplicates

Table: 6. *In vitro* anti-inflammatory activity of *Dolichandrone falcata* (Bovine serum albumin)

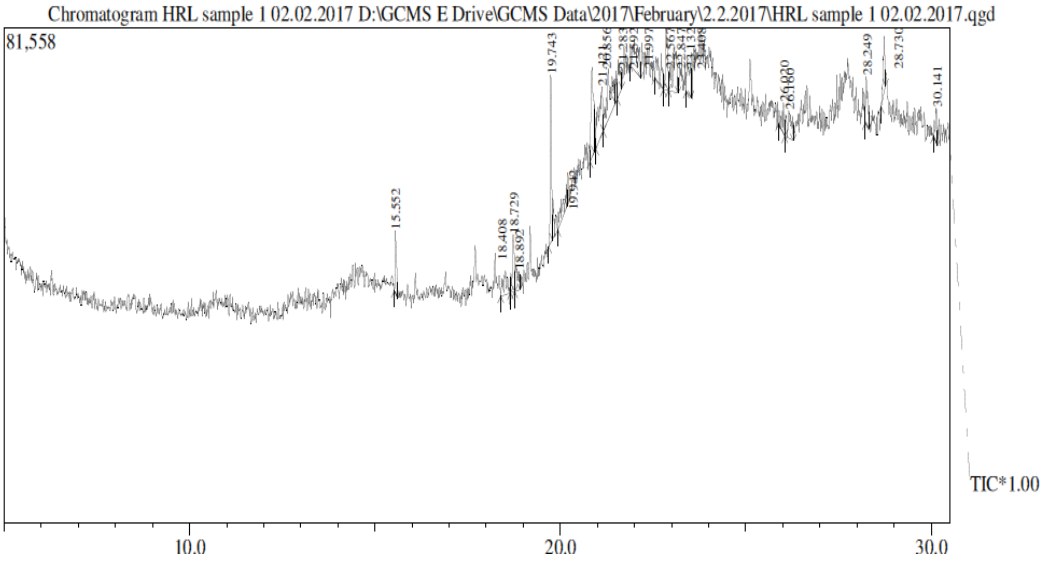
S.No	Doses (µg/ml)	Plant extract	Standard (Std.) (Diclofenac sodium)
1	100	27.89 ± 1.04	19.65±1.65
2	200	44.73 ± 1.04	32.65±2.02
3	300	60.52 ± 1.04	50.23±3.22
4	400	85.96 ± 1.60	75.65±4.47
5	500	91.4 ± 1.60	90.25±5.89

Values are expressed as Mean ± SD for triplicates

Table: 7. *In vitro* anti-inflammatory activity of *Dolichandrone falcata* (HRBC Method)

S.No	Doses (µg/ml)	Plant extract	Standard (Std.) (Diclofenac sodium)
1	100	22.62 ± 1.63	23.56±2.14
2	200	32.83 ± 2.05	41.23±2.68
3	300	52.22 ± 0.77	58.89±3.97
4	400	83 ± 1.55	77.56±5.12
5	500	92.13 ± 0.77	92.32±6.55

Fig :4 .GC- MS analysis of leaves extract of *Dolichandrone falcata*



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