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Phytochemical Screening and Anti inflammatory, Bronchodilator and Antimicrobial activities of the Seeds of *Luffa cylindrica.*

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ABSTRACT

To find out the pytochemical constitutents in the seeds of *Luffa cylindrica* and determine their anti-inflammatory, Bronchodilator and Antimicrobial activity.The fruit of *Luffa cylindrica* collected from the village, Othakadai near Madurai, Tamilnadu, south India were dried and the seeds were separated from the fruit and extracted with petroleumether, benzene, chloroform and alcohol. The petroleum ether extract and benzene extract were mixed and chromatographed, by using solvents n-Hexane, petroleum ether, benzene, ethylacetate and methanol. Finally the compounds isolated Cu-1, Cu-2, Cu-3 and Cu-4. Phytochemical screening was carried out according to standard procedures. Anti inflammatory activity was determined by carragenan induced paw-odema method by using external standard Diclofenoc sodium and oral Brufen. Bronchodilator activity using Guinea pig trachea compared to standard Aminophylline. Anti microbial activity was effective against *S.aureus* and *Candida albicans*. For all the activities the column isolated compounds and alcohol, petroleum ether extracts were used. Sugar, protein, Alkaloids, flavonoids, sterols and glycoside were found to be present in the extracts. Cu-1 has moderate anti-inflammatory and Cu-3 has significant anti-inflammatory activity. Cu-2 and Cu-4 have significant antibacterial and antifungal activity. Cu-4 has significant Bronchodilator activity.*Luffa cylindrica* seed extracts and oil possess good anti inflammatory, Bronchodilator and antimicrobial activity.

Keywords: *Luffa cylindrica,* column extracted oil compounds, anti-inflammatory, Bronchodilator, antimicrobial.

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INTRODUCTION

Most people in the rural areas of the world depend largely on the herbs for the treatment of several ailments because medicinal herbs constitute indispensable components of tradition medicine practice due to low cost, easy assess and ancestral experience [1]. Inflammatory responses are mostly associated with pathological disorders [2]. Although a good number of plant species are used for this purpose , scientific and pharmacological is scare or very little [3]. Bronchial asthma causes considerable financial burdens to nation and individuals. Financial constraints caused due to expense of treatment and curtailed family activities may be break down and disintegration of families [4]. Presence or fear of symptoms may cause asthma patients to avoid physical activities and social situations they might otherwise enjoy. It is difficult to arrive at a physiatrist diagnosis of depression in asthma, although self reports of the conditions are common [5]. Chronic nature of the disease and unpredictability and intensity of acute episodes can substantially lower patients health related quality of life [6]. From 1980s onwards a number of self management programs have been developed and evaluated for pediatric and adult asthma that integrate asthma education with drug therapy as part of a program [7-9]. Despite emerging consensus about the role of physiological factors in asthma morbidity [10], there are very few studies [11,12] addressing very few physiological factors in the management of adult asthma. In India Grover *et al* [13], in a preliminary study addressed physiological factors in management of asthma and found that cognitive behaviour therapy was effective in improving anxiety, depression and quality of life and management of asthma. Present study is an improvement and replication of previous study. In recent years there has been a growing interest to evaluate plants possessing antimicrobial activites for various diseases [14]. A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants [15-17]. Plant derived drugs have become a popular alternative medicine in developing countries. Synthetic antifungal / antibacterial drugs widely used at present are sometimes causing toxicity and adverse drug reactions [18]. Further more, herbal medicines and supplementation are considered less toxic than the synthetic compounds [19]. However, folk medicines have not been studied extensively.

Luffa cylindrica (Linn) M.Roem (Synonym: L.aegyptica ex Hook) family: Cucurbitaceae. The plant is widely distributed through out India. The plant has identified by Dr.Stephen, Dept.of Botany, American College Madurai. The seeds are considered emetic and cathartic. The fruit is used as diuretic and lactogogue [20-24]. The seed oil is reported to be used for skin infections, (wealth of India) in the form of tincture the fruit used in the treatment of ascites, jaundice and billiary and intestinal colitis as also in enlarged spleen and liver. The plant is reputed to have anti tubercular and antiseptic properties. The extract of leaves has been used in snake-bites. The Santals tribesmen use the plant parts in treating convulsions and cramps,

tetanus and also in the treatment of syphilis. The fruit is used in dropsy, nephritis, chronic bronchitis [25-27].

Earlier studies have shown that plant possesses Heptatoprotective [28,29], Anaesthetic activity [30], Antiinflammatory [31], Anthelmintic [32,33], Antimicrobial [34-36], Anticancer [37, 38(a,b)], Enzyme inhibitor [39-44] effects. Constituents previously isolated from the plant (fruits and seeds) crystalline bitter principles from the plants belonging to the family Cucurbitaceae are named Cucurbitacins (A,B,C,D and E) [45] ′α-Elaterin [46], Amarin [47,48], Sapogenins [49], Epicucurbitacin [50,51], Saponins [52], Aminoacid [53], (N-ethyl asparginase, N-Hydroxy methyl asparginase), Bioflavonoids [54], (luteolin iroplumbagin), Flavonoids [55], fatty acids [56], (stearic acid linoleic acid) and Vitamins [57].

At present no known scientific study reported in available literature sources that has been carried out so far an anti-inflammatory, Bronchodilator and anti microbial activity of the plant extract. Thee fore, this study is aimed at exploring the plant (fruit) *Luffa cylindrica* for their therapeutic potential actions.

MATERIALS AND METHODS

Collections of plant materials

The fruits of *Luffa cylindrica* were collected from several plants near Yanamalai-Othakadai, located on the Madurai –Chennai high way during the months of August and September. The stalks were removed and fruits were shade dried for 25 days. The seeds were separated from the fruits and were shade dried for another three days. The dried seeds were made into a coarse powder and were used for different investigations.

Extraction and phytochemical screening

Dried coarse powder of the seeds (250 gms) of *Luffa cylindrica* were placed into the extractor of a Soxhlet apparatus and subjected to extraction by hot percolation method. The extraction was carried out using solvents of increasing polarity starting from petroleum ether, benzene, chloroform and ethanol. The extraction was carried with 2 lit of each solvent for a period of 72to90 hours. At the end of the extraction the respective solvents were concentrated by evaporation. Phytochemical screening was performed using standard procedures [58-60].

Isolation of the compounds by column chromatography

After screening the various extracts obtained from 250 grams of coarse seed powder, the petroleum ether extract and benzene extract were found to be promising. The petroleum ether extract (6.2 gms) was dark green gummy residue. The benzene extract was green viscous residue (21 gms) were mixed and chromatographed over silica gel (100-200mesh).

The column was built up by passing two column volumes of n-hexane before the residue was loaded. The solvent was kept 5 cms above the bed and the residue was carefully loaded in the form of a n-hexane slurry. The column was then developed with a series of solvent starting with n-hexane, petroleum ether, benzene, ethyl acetate and methanol.

The different ratio with succeeding solvent were fixed and fractions of 50 ml were collected up to ethyl acetate-methanol system and there after fractions in smaller volumes were collected, checked with T.L.C. and accordingly pooled, concentrated and processed further. The isolated compounds were named as CU-1, CU-2, Cu-3, and Cu-5.

Toxicity studies [61]

Wistar albino rats (200 ± 50 g) of either sex were kept in the propylene cages and maintained at a temperature of 25 ± 2 °C and had free access to food and water *ad libitu*m. For sub chronic and acute toxicity studies, the rats were divided into two groups of six animals each. First groups served as normal control. Overnight and given water *ad libitum* while food was withheld 3 – 4 hr after oral administration of the n-butanol fraction. The doses of the fraction ranged from 2 -5 g/kg body weight, orally. All animals exhibited normal behavior with no macroscopic changes in viscera.

Evaluation of anti-inflammatory activity [62,63]

Carrageenan induced paw edema in rats

Albino rats weighing approximately 150-200 gms of either sex were divided into five groups of six animals. The dosage of drugs administered is as follows.

Control a control group of rats received orally 5 ml of the normal saline solution.

For oral

Standard drug

The standard group received orally Brufen 40mg/kg body weight.

For external application

Standard drug Diclofenac sodium gel is used as standard drug. It was applied gently to the affected area 3-4 times.

For oral test group

The test group received orally 50mg/kg Cu-3(one of the fraction of the petroleum ether extract.

For external application

Test group

The oils CU-1and CU-2 was applied gently to the affected area 3 to 4 times.All the standard and test for oral and external application were given 30 minutes before the commencement of the study. After that 0.1 ml of 1% w/v carrageen solution in normal solution was injected into the sub plantar tissue of the left hind paw of the rat and right hind paw serves as the reference control. The volume of mercury displaced in the plethysmograph was measured at 0 minutes, 30minutes, 1 hour,2hour,3hour,4hour and 5 hour. The percentage decrease in paw edema of the treated was compared with that of the control and the inhibitory effect of the extracts were studied. The relative potency of the extracts under investigation was calculated, based upon the percentage inhibition of the inflammation.

Percentage inflammation

$$\frac{\text{Volume of control-volume of treated}}{\text{Volume of control}} \times 100$$

Evaluation of Bronchodilator activity

Preparation of tracheal chain: An adult guinea pig was killed by a blow on the head. An incision was made across the neck, the trachea was dissected out. The trachea was cut to make it free from other attachments and transferred to a Petri dish containing Kreb' solution, aerated with a mixture of 95% oxygen and 5%carbondioxide. With the scissors, the tracheal muscle was sectioned transversely between the segments of cartilage, so as to give a number of rings of tracheal muscle 10-12 rings of same width, tied with thread at both end in the form of a chain. One end of the chain was fixed to the glass rod in the bath and other end to the frontal writing lever of twelve fold magnification fore the purpose of recording contraction and dilation on the physiograph. The whole assemble was mounted in an organ bath containing Krebs solution at 37°C under 0.5 gm tension and aerated with the gas mixture. The tissue was allowed to equilibrate for 30 minutes before commencing testing.

Normal contraction of the tracheal chain preparation was recorded. 3 ml of Acetyl choline chloride solution (0.000001%) was added to the organ bath and the contraction was recorded. Contact time of 90sec, and 3 minutes time cycle were kept for proper recording of the responses. Then the tissue was washed out tested

with 32 ml of various extracts of *Luffa cylindrica*, and 2ml (10mg) of aminophylline solution, the dilation produced were recorded on the kymograph. The dilatation produced by the extracts was compared with standard aminophylline.

Evaluation of Anti-microbial activity

Antibacterial activity [64-67]

Assay was carried out by diffusion plate method. The method followed was spread plate technique. The plates free from contamination were spread with 50µl of 48h old culture of bacterial test organism using sterile buds. The standard disc of Ciproflaxacin (sterile) of 5 mm diameter was in the Petri plates. Then the filter paper discs (sterile) of 5mm were soaked in 1ml (1µg/ml) of the test solution and in solvent control DMF. After evaporating the solvent in a sterile atmosphere the drug impregnated discs were placed in Petri plates. The plates were refrigerated for 1h to arrest the growth and for easier diffusion of test compounds. Then the plates were removing from refrigerator and incubated at 37^0C over night is an inverted position. The clear zones of inhibition were measured using Hi media zone reader scale. The values are tabulated. The zones of test solutions were compared with standard Ciproflaxacin.

Antifungal activity

Glucose, peptone and agar were taken in the above proportions and dissolved upto 1000 ml of distilled water. The constituents were heated gently at 100° C with agitation. The pH of the medium was adjusted to 5.4. Then it was transferred to boiling tubes in hot condition and sealed with non-absorbent cotton and sterilized by autoclaving at 121° C (15 lbs pressure) for 15 mts. Then poured aseptically into sterile Petri dishes. The temperature of the medium should not exceed above 50° C when the organisms were inoculated. The standard drug Griseofulvin (10µg/disc) was placed on the media. The sterile whatmann no.2 filter disc (5mm diameter) was soaked in synthesized compounds (200µg/disc) separately and evaporated to dryness and then kept on the media. One more disc immersed in dimethyl formamide and kept on the media as control. The Petri dishes were incubated at 37° C for 24hrs, after placing them in the refrigerator for one hr to facilitate uniform diffusion. Observations were made for the zone of inhibition around the synthesized compounds with that of standard.

Statistical analysis

All the results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by student 't' test [68] at a probability level of $P < 0.001$.

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RESULTS

Preliminary phytochemical screening of all the extracts revealed the presence of sugar, protein, alkaloids, flavonoids, sterols and glycosides as major constituents. Cu-1 is oil has shown more instauration and less acid value. Though it might not have immediate utility as cooking oil its future role as a medicinal compound cannot be overlooked. These observations are based on its highly significant anti fungal, antibacterial and moderate anti-inflammatory activities. This oil has been hydrolyzed and the resulting free fatty acids have been converted into their respective methyl esters for separation on GLC. CU-2 this is the unsaponifiable fraction of the oil. The sterols or related compounds are present in this fraction as the chemical and spectral data suggests. This showed very high antifungal and significant anti bacterial activity. CU-3 has significant anti-inflammatory activity. CU-4 showed bronchodilator activity. This extract showed very high degree of antifungal activity.

DISCUSSION

Carrageenan induced paw edema was taken as a proto type of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator syleaves through a common trigger mechanism. The development of carrageenan induced edema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin [69-71] and the delayed phase is sustained by the leucotrienes and prostaglandins [72]. Flavonoids and tannins are reported to inhibit PG Student synthesis [73]. Most of the NSAIDS have well balanced anti inflammatory and ulcrogenic activities, which are considered to be due to PG synthetase inhibitor activity. The plant extract possess a marked anti-inflammatory activity and hence may pose itself as very good anti-inflammatory drug. Still further investigation with respect to pharmacological and phytochemical profile of the drug needs to be carried out. Three distinct phases are observed during inflammation which are the histamine and serotonin released in the first phase, Kinin and Prostoglandin are released in the second and third phases respectively [74]. Carrageenan induced hind paw oedema in the standard experimental model of acute inflammation. Carrageenan in the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. The extract *Luffa cylindrica* produced significant inhibition of Carrageenan induced paw oedema. The inhibition was however less than that of the standard drug.

The syndrome of bronchial asthma is characterised by wide spread narrowing of the bronchial tree due to contraction of the smooth muscle in response to multiple stimuli resulting in the release of chemical such as histamine. Patients with concurrent obstructive airway disease and coronary artery disease or systemic hypertension need drugs that are free of broncho constricting side effects. Beta blockers have been shown to induce severe asthmatic attacks. Cardioselective beta blockers and angio tensin-converting enzyme inhibitors too have been shown to increase bronchial responsiveness and therefore have to be administered with

caution in such patients. Calcium channel antagonists have shown some protective effect against allergen, histamine, methacholine and exercise induced broncho constriction.

Disc diffusion methods are used extensively to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of the substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. Due to limited capacity of discs, holes or cylinders are preferably used. Most of the bacterial species and the fungal species were inhibited by the plant extract . In this study, one bacterial and fungal species were used to screen the possible antimicrobial activity of the extract. It showed a broad spectrum of activity against all the bacterial strains. Ciproflaxacin and Griseofulvin were used as positive controls for bacteria and fungi, respectively. As reported earlier secondary metabolites like flavonoids, saponins are likely responsible for the observed antibacterial activity of plants [75-77]. The presence of said constituents in the ethanol extract of *Luffa cylindrica* as found in phytochemical test may be responsible for the antibacterial activities.

CONCLUSION

The extracts and isolated compounds (seed oils) of *Luffa cylindrica* has Anti-inflammatory, Bronchodilator and microbial effect of supporting the Ethno pharmacological uses. This effect may be explored in the use of the seeds in the management of CNS and CVS diseases.

ANTI INFLAMMATORY ACTIVITY OF *LUFFA CYLINDRICA*

Sl.No	Drug	Paw volume in ml ±SEM and percentage of inhibition						
		0 Min	30 Min	1 hour	2 hours	3 hours	4 hours	5 hours
1	Control	0.6606 ± 0.0034	0.7362 ± 0.0034	0.8694 ± 0.0034	0.882 ± 0.0046	0.882 ± 0.0062	0.8838 ± 0.00539	0.8992 ± 0.00539
2	Standard(for external)Diclofenac	0.432 ± 0.00294 34.60 %	0.4464 ± 0.00294 39.36 % **	0.5706 ± 0.0034 34.36 % **	0.5418 ± 0.00453 38.57 % **	0.5058 ± 0.00614 42.65 % **	0.4572 ± 0.00465 48.26 % **	0.4482 ± 0.00452 50.10 % **
3	CU-1 (oil)	0.503 ± 0.0061 23.85 %	0.8748 ± 0.00463 18.71 % **	0.7938 ± 0.00452 8.78 % **	0.7308 ± 00463 17.14 % **	0.729 ± 0.0034 17.34 % **	0.7848 ± 0.0029 11.29 % **	0.8802 ± 0.0034 2.026 % *
4.	CU-2 (oil)	0.6282 ± 0.0034 4.93 %	0.7308 ± 0.00463 0.842 % *	0.7434 ± 0.0041 14.53 **	0.8244 ± 0.00462 6.57 % **	0.747 ± 0.00452 15.3 % **	0.738 ± 0.00464 16.49 % **	0.7398 ± 0.0034 17.70 % **

** P < 0.001 – highly significant * P < 0.5 - significant

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2	Standard(for oral) ibrufen	0.4338 ± 0.00614 34.33 %	0.4536 ± 0.00509 38.38 % **	0.5868 ± 0.00465 32.50 % **	0.5436 ± 0.00465 38.36 % **	0.5238 ± 0.00614 40.61 % **	0.4536 ± 0.00294 48.67 % **	0.4248 ± 0.00294 52.70 % **
3	CU-3-crystals	0.5958 ± 0.0034 9.93 %	0.8028 ± 0.00464 8.93 % *	0.6786 ± 0.0034 22.10 % **	0.6084 ± 0.00465 31.06 % **	0.8154 ± 0.0034 7.59 % *	0.6804 ± 0.00464 23.05 % **	0.657 ± 0.00452 26.85 % **

** P < 0.001 – highly significant, * P < 0.5 - significant

BRONCHODILATOR ACTIVITY

Sl.No	Drug/Extract	Height in cm	Height difference with control(cm)	% activity in relation to Aminophylline
1	Acetyl choline	2.1	-	-
2	Aminophylline(std)	0.6	1.5	100%
3	Alcoholic extract	1.2	0.9	60%
4	CU-1(oil)	1.4	0.7	46.6

ANTI BACTERIAL ACTIVITY

Sl.No	Sample	Zone of inhibition	Percentage inhibition
1	Ciprofloxcin(5mcg/ml)	2.5	100
2	Alcoholic extract	1.5	60
3	Ether extract	1.0	40
4	Cu-1 (oil)	3.1	124
5	CU-2(oil)	1.5	60

ANTI FUNGAL ACTIVITY

Sl.No	Sample	Zone of inhibition	Percentage inhibition
1	Ciprofloxcin(1%)	5	100
2	Cu-1 (oil)	11	220
3	CU-NS	--	Very high
4	Alcohol extract	12	240

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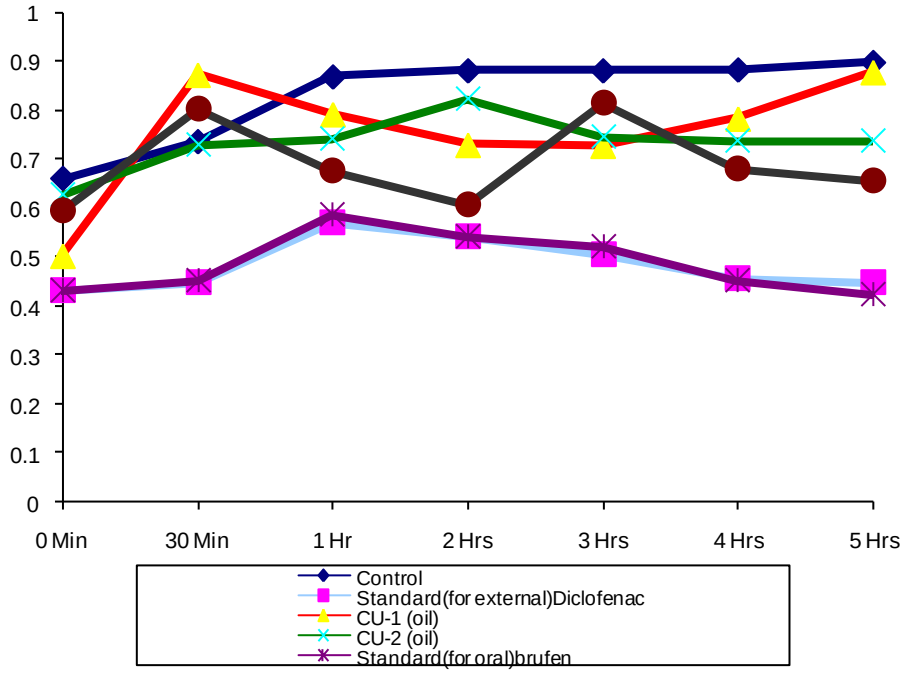

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