

**Detection and Estimation of Phenolic Acid and Flavonoids in Leaves of *Cadaba indica* Lam by High Performance Thin Layer Chromatography**Thirumalai V<sup>1</sup>, Nirmala P<sup>1\*</sup>, Venkatanarayanan R<sup>2</sup><sup>1</sup>Department of Pharmacology, RMMC, Annamalai University, Tamilnadu, India<sup>2</sup>RVS College of Pharmaceutical sciences, Coimbatore, Tamilnadu, India**Original Research Article****\*Corresponding author**

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**Abstract:** *Cadaba indica* Lam. is one of the medicinal plants used in Indian traditional systems of medicine for the treatment of various diseases of mankind. The present study mainly aimed to estimate the content of major constituent flavonoid present in various extracts of leaves of *Cadaba indica* collected from Tamilnadu. HPTLC method was adopted for determining the content of the active constituent present in the various extracts using marker compound Quercetin, rutin and gallic acid. The HPTLC method was performed using HPTLC aluminium sheets precoated with silica gel 60 GF<sub>254</sub> as stationary phase and mobile phase as toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4). The developed chromatogram was scanned at 254 nm using Camag scanner III. The chromatogram obtained and the peak profile of the components collected by scanning could be made up the fingerprint of the various extracts of *Cadaba indica* leaves compared with antioxidants markers of flavonoids and phenolic acids. HPTLC fingerprint analysis showed that the presence of rutin, quercetin and gallic acid which may be responsible for the anti-oxidant activity mentioned. This method is appropriate to determine the percentage of flavonoids and phenolic acids in various extracts of leaves of *Cadaba indica*.

**Keywords:** *Cadaba indica* Lam., HPTLC, rutin, quercetin, gallic acid.

**INTRODUCTION**

Traditional medicine is widely used for prevention, diagnosis and treatment of physical and mental illness [1]. World population of 80% people depends on herbal medicine for their treatment for the reason that of the high cost and adverse effects of accessible synthetic drugs. Traditional medicine system is preferred due to its affordable, accessible, safety and traditional faith. In such a case the quality assurance, standardization and validation is essential to achieve the safety and efficacy of the traditional medicines [2]. The therapeutic efficiency of the drugs depends greatly on the use of proper and genuine raw materials. Because of this, the guarantee of safety, quality and subsequent efficacy of the medicinal plants and herbal products have now become a major and explanation area under discussion, so the standardization of plant material is compulsory [3]. High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs due to its simplicity, high sensitivity, accuracy and less expensive [4]. *Cadaba indica* Lam. (Capparidaceae) is widely distributed in arid area worldwide. *C. indica* Lam. is an unarmed, branched shrub up to 3m height. Leaves are simple,

elliptic-oblong, size 12-15 by 8-12mm, dull green in colour. Flower is greenish white in few flowered terminal corymbs, fruit berry and cylindrical Toulouse. It is commonly distributed in scrub jungles and rocky areas (tropical and sub-tropical). November to April is the Flowering and fruiting season. *Cadaba indica* Lam. is a traditional plant medicine which was first described in the book named "Jala thirattu" by PuliPani sidhar. The plant was traditionally used for the treatment of syphilis, sores and as an antiphlogistic, deobstruent, emmenagogue and anthelmintic. Leaf juice is specially used as a remedy for dysentery, stimulant, purgative, fever, cough, and lung problem [5,6]. The *Cadaba indica* Lam. leaves contain steroids, lactones, flavonoids, phenols, reducing sugar and tannins [6-8]. The methanol leaf extract of *Cadaba indica* contained sensible amount of flavonoid and total phenolic compounds which are potent antioxidant agents to cure oxidative distress [8]. Alcoholic extract of *Cadaba indica* plant has good Anti-inflammatory, analgesic and antimicrobial properties [6-9]. The present study was to detect and estimate the percentage of rutin, quercetin and gallic acid which are important markers of flavonoids and phenolic acids in soxhlet-hot

percolation and cold maceration methonolic leaf extracts of *Cadaba indica* Lam.

## MATERIALS AND METHODS

### Collection of plant materials

The plant *Cadaba indica* Lam. was collected during flowering season in February, 2017 from, Melur, Madurai district, Tamil Nadu, India. The plant was authenticated by Mr.V.Chelladurai Research Officer of Botany, Central Council for Ayurveda and Siddha, Government of India. A voucher specimen was preserved in our laboratory for future reference.

### Preparation of extract

#### Hot percolation method

100g of Coarse leaf powder of *Cadaba indica* Lam. was subjected for extraction process using soxhlet apparatus. The coarse powders of the leaves were first extracted with petroleum ether (*Cadaba indica* hot petroleum ether extract-CIHPEE), and marc was dried and defatted material is again extracted with methanol for 72 hours. Named as *Cadaba indica* hot maceration methonolic extract (CIHME).

#### Cold maceration method

100 g of the *Cadaba indica* Lam. leaf powder was first extracted with petroleum ether by soaking in the solvent for 72 hours (*Cadaba indica* cold maceration petroleum ether extract -CICPEE) and the defatted material was extracted with methanol (*Cadaba indica* cold maceration methanol extract – CICME).

#### Decoction method

The decoction method is carried out to prepare aqueous extract from 100 g of coarse leaf powder of *Cadaba indica* Lam. in distilled water.

The extracts were then filtered and the solvents were evaporated to dryness under reduced pressure in Eyele Rotary evaporator (Japan) at room temperature to give a viscous mass. The obtained crude extracts were weighed and stored at 4°C for the further analysis.

### Chemicals and Instruments

Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. TLC was carried out using Merck aluminum sheet coated with silica gel GF<sub>254</sub> (0.2 mm).

### Sample application

10µl of test solutions and 5µl of standard solution were loaded as 6mm band length in the 10 x 10 Silica gel 60F<sub>254</sub>TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

### Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (standards

and the plate was developed in mobile phase up to 80mm.

### Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was Photo documented the images at UV 254nm.

### Scanning

The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version A. The percentage of active constituents present in various extracts of *Cadaba indica* leaves were compared with that of standard markers. The HPTLC image is shown in Figure1.

## RESULTS

The following different solvent compositions were tried for monitoring the elution of components in various extracts. Ethyl Acetate: Methanol: Water Toluene (100:15.5:13.5:2), Toluene: ethyl acetate (93:7) Ethyl acetate: glacial acetic acid: formic acid: water (100:3:3:28), Ethyl Acetate: Methanol: Chloroform (6:4:0.3).Ethyl acetate: methanol: water (100:15.5:13.5), Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4). Totally 6 mobile phases were trailed for better elution of formulations. Of which Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) were given better elution for all the extracts to screen in one plate. The optimized chamber saturation time for mobile phase was 3.0 min at room temperature (25 ± 1°C). The densitometry analysis was performed at 254 nm in absorbance mode. The elution of both the extracts were carried out in mobile phase of toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) and in this mobile phase elution was good results were tabulated by considering every R<sub>f</sub> value as one ingredients of extracts whether it may be pharmacologically active or inert but for screening the number of principle in the extracts can be considered as one of the principle in it. Therefore the obtained R<sub>f</sub> value were compared with R<sub>f</sub> value of the standards and well known free radical scavengers rutin, quercetin and gallic acid in various extracts of *Cadaba indica* leaves (Table :2). For identifying these free radical scavengers' rutin, quercetin and gallic acid, we used UV light at 254 nm. Separation of hot macerated Petroleum ether extract (CIHPEE) in chromatogram (10 µl) showed three components at three fourth distance of the developed plate among one is equal to R<sub>f</sub> value of quercetin (Fig: 2). Hot maceration (soxhlet) methonolic extract (10 µl) showed traces of rutin (Fig: 3). The cold macerated petroleum ether extract in chromatogram showed six components and none of them were similar to the standard markers (Fig: 4). Partition of Cold Maceration methonolic leaf extract (10 µl) chromatogram (Fig: 5) showed the presence of rutin, gallic acid and quercetin. *Cadaba indica* Hot aqueous extract chromatogram showed rutin and quercetin traces

(Fig: 6). Fig: 7, 8 and 9 represents the chromatogram of standard marker rutin, gallic acid and quercetin respectively.

#### HPTLC screening of CIHPPEE

Petroleum ether leaf extract of *Cadaba indica* (Hot maceration – soxhlet method) chromatogram showed 3 peaks (Fig .1). *R<sub>f</sub>* value of the third peak (0.86) coincided with standard quercetin *R<sub>f</sub>* value (0.85) and the peak area was 9780.6 (Table .2). Quercetin was found to be 0.49% w/w.

#### HPTLC screening of CIHME

*Cadaba indica* Hot maceration methanolic extract (CIHME) showed 9 peaks (fig.2), the second peak *R<sub>f</sub>* value (0.17) coincided with the *R<sub>f</sub>* value of Rutin standard and its peak area was 4814.3 (Table.2). The percentage of Rutin was found to be 0.206 % w/w. The eighth peak *Cadaba indica* cold maceration (0.87) coincided with quercetin *R<sub>f</sub>* value. The peak area was 2885.0 (Table.2) and it was found to be 0.141% w/w.

#### HPTLC screening of CICPEE

Cold maceration petroleum ether extract (CICPEE) showed 6 peaks (fig.3). The *R<sub>f</sub>* values of all

the six peaks did not coincide with the standard markers.

#### HPTLC screening of CICME

*Cadaba indica* cold maceration methanolic extract (CICME) showed 8 peaks (fig.4), the second, seventh and eighth peaks coincided with the standard markers rutin, gallic acid and quercetin respectively. The second peak *R<sub>f</sub>* value was 0.17 and peak area was 5155.2 (Table.2) the percentage of Rutin was found to be 0.221% w/w. The seventh peak *R<sub>f</sub>* value was 0.76 and peak area was 5213.2 (Table.2) the percentage of gallic acid was 0.198% w/w. The eighth peak coincided with quercetin *R<sub>f</sub>* value as 0.84 and peak area was 18807.2, the estimated percentage was 0.94% w/w. (Table.2)

#### HPTLC screening of CIHAE

Hot aqueous extract of *Cadaba indica* leaf peaked 10 times in chromatogram (fig.5). The third and tenth peak *R<sub>f</sub>* values coincided with the *R<sub>f</sub>* values of rutin and quercetin respectively. The third peak *R<sub>f</sub>* value of 0.16 matched the *R<sub>f</sub>* value of the standard rutin and flavonoid percentage in aqueous extract was estimated to be 0.089% (table .1). The tenth peak *R<sub>f</sub>* value 0.84 coincided with the standard quercetin and to be estimated flavonoid percentage was 0.49% (table.2).

Table-1: yield of *Cadaba indica* Lam. leaf extracts

Extract type	Yield ( %)
CIHPPEE	1 %
CIHME	15.82 %
CICPEE	7.5 %
CICME	6.2 %
CIHAE	31.7 %

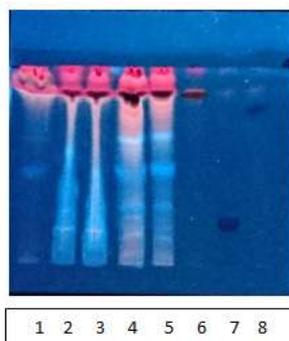


Fig-1: chromatogram of five various extracts of *Cadaba indica* Lam and three standards after development in mobile phase

1. *Cadaba indica* hot petroleum ether extract (CIHPPEE), 2. *Cadaba indica* hot maceration methanol extract (CIHME), 3. *Cadaba indica* cold petroleum ether extract (CICPEE), 4. *Cadaba indica* cold maceration methanol extract (CICME), 5.

*Cadaba indica* hot aqueous extract (CIHAE), 6. Standard reference Quercetin, 7. Standard reference Rutin, 8. Standard reference Gallic acid

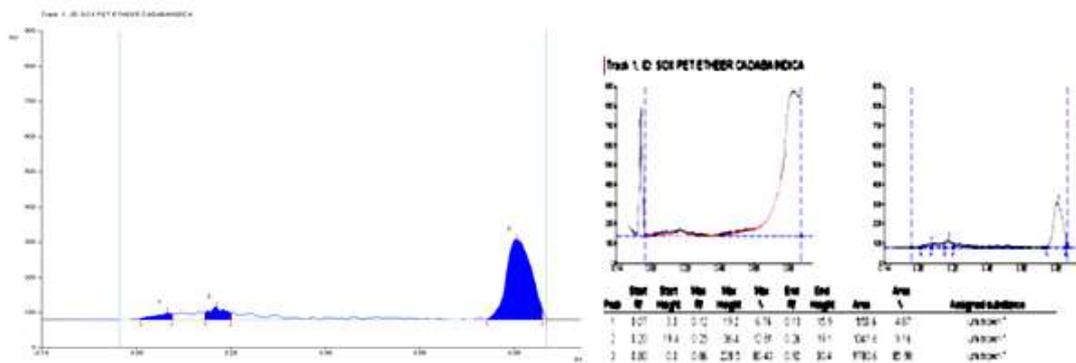


Fig-2: Chromatogram of *Cadaba indica* hot petroleum ether extracts (CIHPEE) track-1

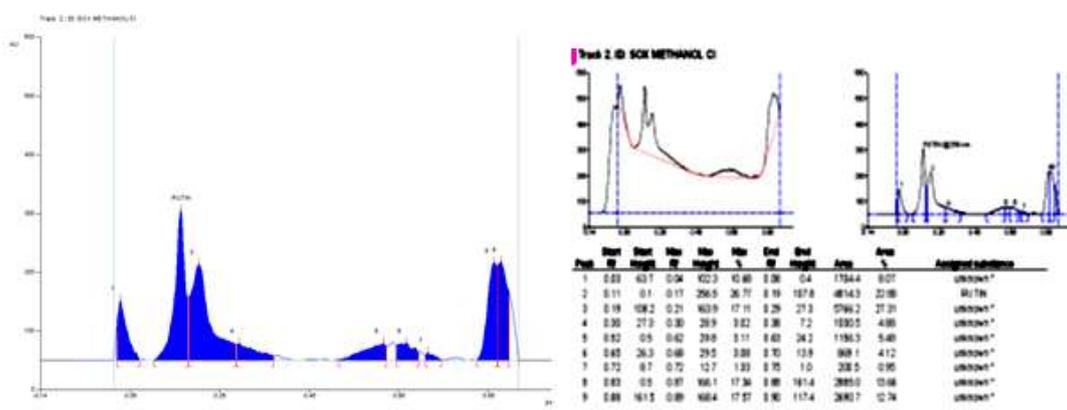


Fig-3: Chromatogram of *Cadaba indica* hot maceration methanolic extracts (CIHME) track-2

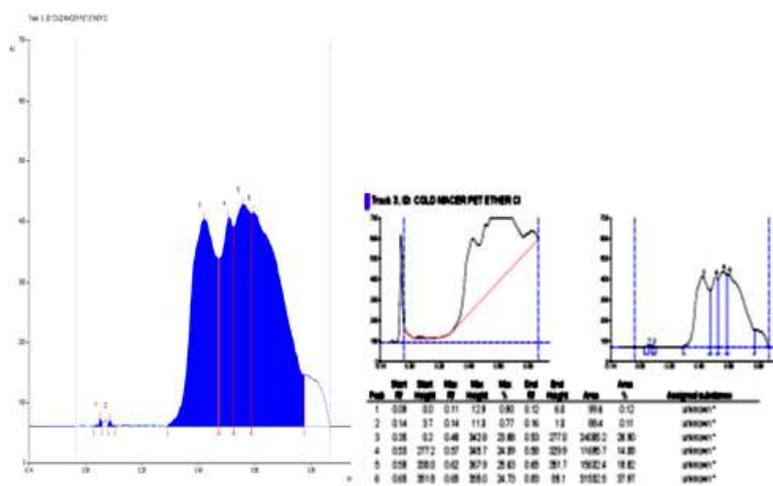


Fig-4: Chromatogram of *Cadaba indica* cold petroleum ether extract (CICPEE) track-3

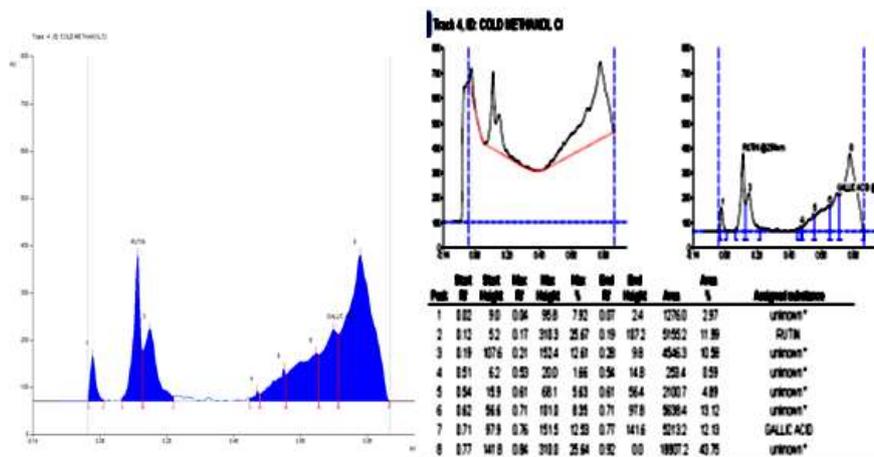


Fig-5: Chromatogram of *Cadaba indica* cold maceration methanol extract (CICME) track-4

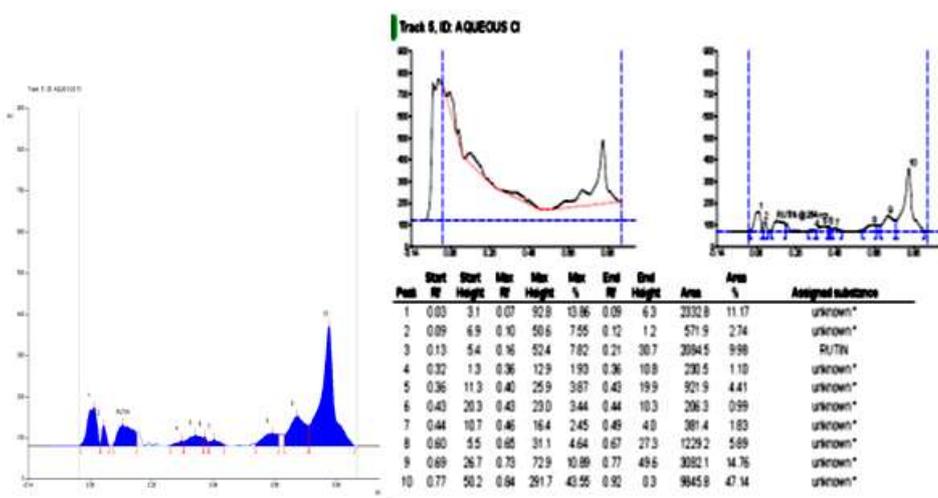


Fig-6: Chromatogram of *Cadaba indica* hot aqueous extract (CIHAE) track-5

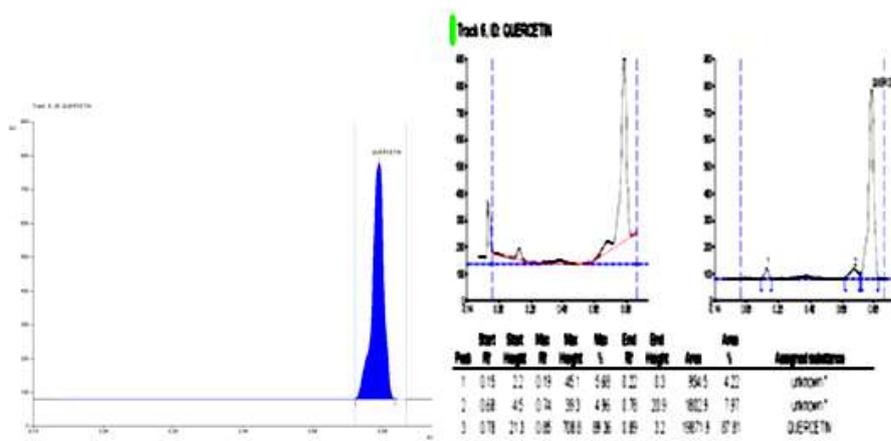


Fig-7: Chromatogram of standard Quercetin track-6

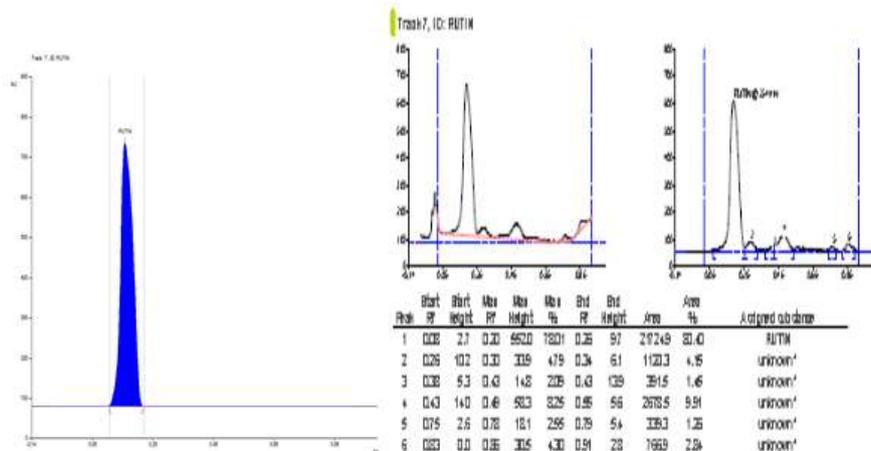


Fig-8: Chromatogram of standard Rutin track-

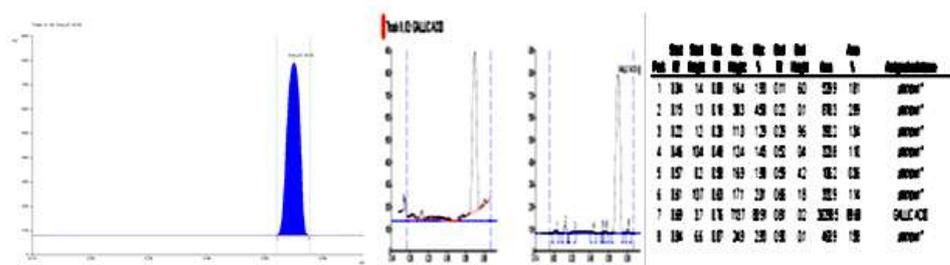


Fig-9: Chromatogram of standard Gallic acid track-8

Table-2: Rf values of standard markers and various extracts of *Cadaba indica* Lam. leaves

Track no	Name of the extract	No of compounds present	Rf value of the compounds in extract	Presence of markers in extract	Area of peak	Amount of marker present µg/10 µl	Percentage of marker in extract
1	CIHPEE 10 µl	3	0.86	Quercetin	9780.6	4.92 µg	0.492%
2	CIHME 10 µl	9	0.17	Rutin	4814.3	2.06 µg	0.206%
			0.87	Quercetin	2885.0	1.41 µg	0.141%
3	CICPEE 10 µl	6	-	-	-	-	-
4	CICME 10 µl	8	0.17	Rutin	5155.2	2.21µg	0.221%
			0.76	Gallic acid	5213.2	1.98µg	0.198%
			0.84	Quercetin	18807.2	9.464 µg	0.946%
5	CIHAE 10 µl	10	0.16	Rutin	2084.5	0.891µg	0.0891%
			0.84	Quercetin	9845.8	4.95µg	0.495%
6	STD Quercetin 5 µl	1	0.85	Quercetin	19871.9	5 µg	100%
7	STD Rutin 5µl	1	0.16	Rutin	23373.8	5 µg	100%
8	STD Gallic acid 5 µl	1	0.76	Gallic acid	26298.5	5 µg	100%

## DISCUSSION

Quantification of an individual compound with HPTLC is an approved and a reliable method. *Cadaba indica* leaf extracts were prepared with various solvents like petroleum ether, methanol and distilled water (aqueous extract). Methonolic extract was prepared in two different methods as hot percolation by soxhlet apparatus and cold maceration. Hot percolation soxhlet extraction is one of the standard methods of extracting oil based compounds from the leaves and this method yield more extract than cold maceration method [10]. Mohan *et al.* reported that total phenol and flavonoids are more in methonolic extract of *Cadaba indica* among the other solvent extracts [8]. 100 g of powdered leaves yielded 15.8g of extract from soxhlet extraction, while 100g of powdered leaves in cold maceration method yielded 6.2g during our extraction too. Mohan et al also reported that the anti-oxidant activity is more reliable with methonolic extract of *Cadaba indica* [8]. Flavonoids are potent anti-oxidants and have protective action against inflammation, allergy, ulcer and microbial infection [16-18]. In our present study we found that cold maceration methonolic leaf extract of *Cadaba indica* comprised of the marker flavonoids rutin, quercetin and gallic acid. Previous studies reported that this plant possessed anti-inflammatory, analgesic and anti-oxidant activity [6-9]. In this study too, HPTLC finger print analysis showed the presence of rutin, quercetin and gallic acid which may responsible for the above mentioned activities. Rutin was reported to be a free radical scavenger and anti-inflammatory agent [19, 20]. Rutin also suppresses cellular immunity [21]. Quercetin has been reported to be an anti-allergic and anti-inflammatory agent [22]. Gallic acid is known to have anti-oxidant, anti-inflammatory, anti-cancer, and anti-microbial property [23-25].

## CONCLUSION

The constituents of *Cadaba indica* Lam. is mainly responsible for its pharmacological actions. The availability and percentage of phytoconstituents may differ according to the method and solvent system of extraction. Its anti-inflammatory, anti-allergic and immune suppression activity may be due to the presence of flavonoids and phenolic acid. In our study both types of methonolic extract of *Cadaba indica* Lam. through high performance thin layer chromatography yielded rutin and quercetin. The cold maceration methonolic leaf extract of *Cadaba indica* Lam. was found to be possessed gallic acid in addition to rutin and quercetin. The percentage of rutin and quercetin was more in cold maceration methonolic leaf extract. We concluded that recent HPTLC method may be adopted for routine detection and simultaneous estimation of rutin, quercetin and gallic acid in various extracts of *Cadaba indica* Lam. In addition in our study, we found that the cold maceration method of extraction may yield more active constituents than the hot percolation (soxhlet) method.

## CONFLICT OF INTERESTS

We declare that we have no conflict of interest regarding this publication.

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