

Original article

Effect of cultural condition on element contents in raw material vis-a-vis impact of solvent nature on estimation of phytochemicals and screening of anthelmintic activity of *Melia dubia* Cav. leaf

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Abstract

A comparative study was conducted to reveal the anthelmintic activity potential of aqueous and methanol leaves extracts of *Melia dubia* Cav. (MDC), collected from four different demographical locations of India, viz., West Bengal, Karnataka, Kerala and Tamil Nadu. Preliminary soil nature was analyzed as per the standard methods and elemental analysis for raw leaf samples was carried out by atomic absorption spectrophotometer which revealed safety use of raw materials for further study. Thereafter, preliminary phytochemical screening of aqueous leaf extracts (collected from all the zones) showed the presence of flavonoids, glycoside, alkaloids, phenols, carbohydrates whereas alkaloids, phenols, flavonoids, steroids, tannins, carbohydrate and proteins are present in methanol leaf extracts. Based on the results, total phenolic and total tannin contents were estimated by Folin-ciocalteu method where gallic acid was used as standard. Chloride colorimetric method was applied for total alkaloid content where atropine used as a standard. The result showed increased in total phenol and total tannins content (102.13 ± 0.01 mg and 64.24 ± 0.13 mg of gallic acid equivalents, respectively) and alkaloids content (82.71 ± 0.12 as mg of atropine equivalents) in methanol leaf extract collected from West Bengal zone (soil pH 6.32 ± 0.01), followed by Kerala zone (99.26 ± 0.01 mg for phenolics content, 58.36 ± 0.01 mg for tannin and 78.86 ± 0.01 mg for total alkaloids) where soil was pH 6.48 ± 0.11 . Furthermore, the anthelmintic activity was carried out against *Pheretima posthuma* (Earthworms) at varied concentrations of 25, 50, 100 and 150 mg/ml and compared with standard albendazole (25 and 50 mg/ml) and distilled water as control. Both the extracts exhibited concentration dependent paralytic effect, followed by death on the test organism. Among the zones, methanol and aqueous extracts from West Bengal zone showed highest paralytic activity against the test organism (paralysis at 6.47 and 10.3 min, followed by death at 9.42 and 16.27 min, respectively at 150 mg/ml) and the effects may be due to high content of phenolics, tannins and alkaloids in methanol leaf extract of MDC. Finally concluded that MDC leaf has powerful anthelmintic activity and proved as a novel source of antiparasitic drug.

Key words: *Melia dubia* Cav., anthelmintic, geographic zones, extracts, phytochemical study

1. Introduction

It is known to us that higher plants are novel sources for development of lead compounds and drug discovery. Therefore, a vast percentage of the world populations (more than 80%) have faith on herbal medicines for their primary health care needs (Valentina *et al.*, 2013), and about 85 per cent of traditional medicines involve the use of plant extracts due to lower side effects than synthetic drugs (Murthy *et al.*, 2005). Traditional folk remedies from plants have always showed the path to the scientists to search for new medications and newer drug molecules in order to maintain and promote healthy life against parasitic worms. Over two billion

people are suffering from parasitic worm infections reported by the World Health Organization (Mulla *et al.*, 2010) and is estimated by the year 2025, about 57% of the population will be influenced by this infection which will be one of the major health problem in the developing countries (Clewes and Shaw, 2000). Infection with parasitic worms is known as helminthiasis which is common infectious agents of humans and humans are the reason for spread of these pathogens to uninvolved populations through travel, migration and military operations as a result lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis occurs. Despite the prevalence of parasitic infections, there are scanty researches on anthelmintic drugs due to increasing resistance towards worms (Sondhi *et al.*, 1994) and therefore alternative strategies against those parasitic worms are most essential. Looking at that the therapies with natural plant products is one of the major options to control these pathologies infected by those worms', viz. pinworm, roundworm, or tapeworm. Hence thorough screening is required to establish genuine plant drug for their anthelmintic activity. Several researchers have reported

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the anthelmintic activity of plant parts like *Buchholzia coriacea* and *Gynandropsis gynandra* methanolic leaves and stem extracts (Ajaiyeoba *et al.*, 2001), crude latex of *Calotropis procera* (Shivkar and Kumar, 2003), rind of *Punica granatum* ethanol extract (Lalhmingshuanmawii *et al.*, 2014), aqueous and ethanolic stem extract of *Tinospora cordifolia* (Tiwari *et al.*, 2011), *etc.*, on helminthes. Based on that, the present study was undertaken to evaluate state wise available MDC plant leaf for anthelmintic activity. Thereafter, cultural conditions also play an essential role for percentage content of constituents in plant parts vis-a-vis content of elements in plant parts and the same was evident of earlier research papers, *viz.*, soil acidity and season are factors affecting mineral uptake by plants because these minerals are used by plants as structural components in carbohydrates and proteins, magnesium in chlorophyll and phosphorus in energy production, enzyme activators like potassium, zinc is for stimulate the immune system, zalcium helps in firmness of fruits (Olaiya, 2006; Soetan *et al.*, 2010; Nadim *et al.*, 2011) and also influenced by agronomic practices, environmental factors and other related soil factors (Nepovim *et al.*, 1998; Ashraf *et al.*, 2006; Sekeroglu and Ozguven, 2006; Ozguven *et al.*, 2008; Das *et al.*, 2012). Thereafter, effect of heavy metals should not be ignored because content of heavy metals in raw materials if is more than acceptable limit then several health hazards are developed and, hence thorough standardization of the raw materials are always essential before performing any therapeutic activities. Many literatures reported that influence of soil nature on heavy metal contents in raw plant materials (Das *et al.*, 2011; Moscow and Jothivenkatachalam, 2012; Singh *et al.*, 2014). Furthermore, effect of the constituents on therapeutic activity is also dependent on various factors like geographical location, soil nature and agroclimatic nature and many scientific researches evident for the same (Orhan *et al.*, 2013; Das and Trivedi, 2015). Based on that, MDC plant was selected for the present investigation which was collected from four different cultural zones. These zones were selected based on the climatic variation and differ in soil nature. As a background of this plant, is belongs to Meliaceae family and has important secondary phytoconstituents. MDC is a Indian forest plant, and due to its various potentiality, it become economically important plant. Hence, the importance of this plant can't be ignored in the field of medication. As reviewed for its therapeutic applications, every part of the plant is being used as a source of traditional medicines, *viz.*, treatment of leprosy, eczema, asthma, malaria, fevers, cholelithiasis, acariasis and pain (Kokwaro, 1976; Govindachari, 1992). Specifically, the fruits of MDC are used in skin diseases and also as an anthelmintic (Purushothaman *et al.*, 1984). Different leaf extracts of MDC was evaluated for various pharmacological activities, namely; growth inhibitory activity (Koul *et al.*, 2000), microbiocidal and mosquito larvicidal activities (Chanthuru *et al.*, 2014), antiurolithiatic activity (Vennila and Marthal Mariyal, 2015), *etc.* These activities mainly depend on the phytoconstituents that are present in MDC. It was revealed that the plant is rich source of bioactive limonoids (highly oxygenated and modified terpenoids) and because of that it shows insecticidal, insect antifeedant, anticancer, antiviral activities (Endo *et al.*, 2002; Koul *et al.*, 2004; Awang *et al.*, 2007). Apart from, it contents alkaloids, carbohydrates, steroids, tannins, flavonoids, saponins glycosides (Valentina *et al.*, 2013), diterpenes and sesquiterpenes,

antioxidants, phenolic derivatives and lipid compounds (Murugesan *et al.*, 2013). The present investigation was carried out first time to reveal an impact of geographical conditions, soil nature on elemental content in raw leaves, estimation of various phytoconstituents vis-a-vis in relation to its anthelmintic activity that correlate with the state wise collection of the leaf samples.

2. Materials and Methods

2.1 Study area

Kolkata of West Bengal state (Latitude: 22°32'N and Longitude: 88°19'E), from Bangalore in the state of Karnataka (Latitude: 12°58'38 N and Longitude: 77°35'14 E), Ooty in the state of Tamil Nadu (Latitude: 11° 24' 0" N and Longitude: 76° 42' 0" E) and Palakkad in the state of Kerala (Latitude: 10° 46' 21" N and Longitude: 76° 39' 5" E) were selected (Figure 1). Kolkata climate is a blend of coastal and inland elements common to West Bengal and the temperature has a range between 12°C and 38°C. The city has sandy loam soil with pH 6.30 to 6.40. Bangalore has a tropical climate with distinct dry seasons. The temperature of Bangalore has a relatively narrow range between 13°C and 36°C. Bangalore soil nature is red laterite with pH 7.20 to 7.40. Ooty (Tamil Nadu) climate is cold with average temperature of 12°C to 27°C with soil nature of loose lateritic loam (pH 3.70 to 6.10) and thereafter Palakkad plain area of Kerala has red loam soil with pH 5.80 to 6.30 and average temperature ranges from 22°C to 37°C.

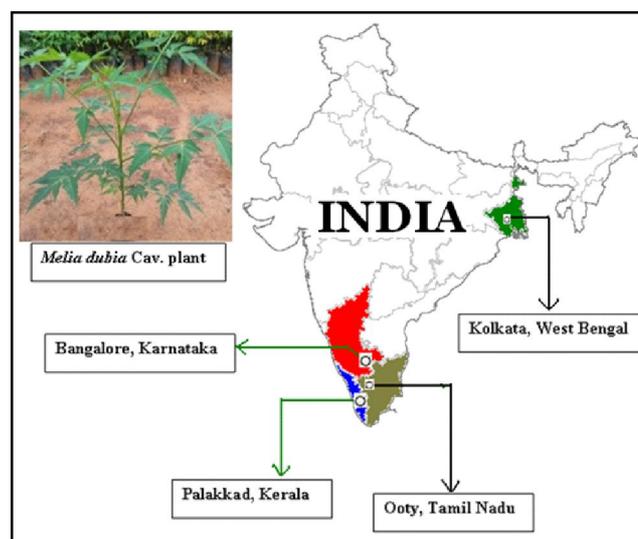


Figure 1: The map showing the locations of *Melia dubia* Cav. growing regions in India from where soil samples and leaf samples were collected from four different States

2.2 Analysis of soil sample

Soil samples were collected at the depth of 8-10 cm, using stainless steel soil augers from the locations where plant samplings were made. Sample IDs of collected soils have been given as WB, KAR, TN and KL for West Bengal, Karnataka, Tamil Nadu and Kerala, respectively. The pH was measured with pH meter, using equal ratio of soil and water and electrical conductivity (EC) was measured with a Electroconductometer and probe as using a 1:5 soil and water ratio (McLean,1982). The percentages of sand, silt and clay were determined with International pipette method (Piper, 1950).

Determinations of cation exchange capacity (CEC) were made in BaCl_2 by the Gilman method (Rhoades, 1982) and total organic carbon (C) was determined by wet dichromate oxidation method (Nelson and Sommers, 1982). Thereafter, total metals and DTPA extractable metals (iron: Fe; copper: Cu; zinc: Zn; lead: Pb; cadmium: Cd; nickel: Ni; Arsenic: As and chromium: Cr) were determined with the help of Atomic Absorption Spectrophotometer (AAS, Perkin Elmer model: AAnalyst 100; Australia) by acid digestion method. 10 g of soil sample was taken in a conical flask and 20 ml

of 0.005 M DTPA (0.005 M DTPA; 0.1 M Triethanol amine and 0.01 M CaCl_2 , 2 H_2O) was added to it. Then it was shaken for 2 hours on a mechanical shaker and it was filtered with Whatman No. 42 filter paper. Then the filtrate was determined for various metal contents in different soils. Blank samples were also prepared for corrections. All the samples were checked by carrying out triplicate analyses for the reproducibility of the method used. The mean value of concentrations for each element has been reported along with standard deviation. The results of relevant geochemical properties of soils were tabulated in Table 1 and Figure 2.

Table 1: Geochemical properties of the collected soils (values represent mean of three replications \pm SE, same letter(s) in a particular row represent non-significant difference between the samples)

Various soil parameters	Location with soil sample ID			
	Kolkata, WB	Bangalore, KAR	Palakkad, KL	Ooty, TN
pH	6.32 \pm 0.14 ^a	7.33 \pm 0.06 ^b	6.48 \pm 0.02	5.43 \pm 0.11
EC (1:5) (mScm ⁻¹)	11.10 \pm 0.11 ^b	15.20 \pm 0.23 ^b	13.09 \pm 0.03 ^a	12.46 \pm 0.21 ^b
Texture	sandy loam soil	red laterite	red loam soil	loose lateritic loam
CEC (cmol kg ⁻¹)	18.68 \pm 0.31 ^b	11.20 \pm 0.12 ^a	15.92 \pm 0.03 ^a	14.48 \pm 0.22 ^b
Total metals (mg kg⁻¹)				
Cd	2.10 \pm 0.10 ^a	7.20 \pm 0.06 ^a	2.33 \pm 0.03 ^a	4.65 \pm 0.24 ^b
Cr	3.25 \pm 0.13 ^b	6.40 \pm 0.16 ^b	3.76 \pm 0.26 ^b	5.98 \pm 0.16 ^b
Cu	11.33 \pm 0.11 ^a	10.30 \pm 0.12 ^a	12.96 \pm 0.14 ^b	12.22 \pm 0.01 ^a
Fe	82.65 \pm 0.23 ^b	72.70 \pm 0.62 ^a	78.54 \pm 0.21 ^b	76.67 \pm 0.24 ^b
Ni	4.30 \pm 0.20 ^b	8.80 \pm 0.47 ^a	6.88 \pm 0.01 ^a	7.44 \pm 0.21 ^b
Pb	2.85 \pm 0.10 ^b	11.55 \pm 0.47 ^b	5.78 \pm 0.22 ^b	10.55 \pm 0.22 ^b
Zn	26.28 \pm 0.22 ^b	18.20 \pm 0.24 ^a	20.56 \pm 0.12 ^b	19.30 \pm 0.14 ^b
DTPA-extractable metals (mg kg⁻¹)				
Cd	0.29 \pm 0.04 ^b	0.76 \pm 0.01 ^a	0.78 \pm 0.01 ^a	0.88 \pm 0.03 ^b
Cr	0.54 \pm 0.20 ^b	0.84 \pm 0.01 ^b	0.71 \pm 0.04 ^b	0.76 \pm 0.02 ^b
Cu	1.12 \pm 0.01 ^a	1.02 \pm 0.02 ^a	1.39 \pm 0.05 ^b	1.51 \pm 0.01 ^a
Fe	8.96 \pm 0.06 ^b	18.64 \pm 0.07 ^a	19.03 \pm 0.11 ^b	18.55 \pm 0.21 ^b
Ni	0.78 \pm 0.01 ^a	1.96 \pm 0.04 ^a	0.98 \pm 0.21 ^b	1.10 \pm 0.01 ^a
Pb	0.43 \pm 0.03 ^b	2.98 \pm 0.06 ^b	0.78 \pm 0.20 ^b	0.89 \pm 0.04 ^b
Zn	1.98 \pm 0.21 ^b	0.48 \pm 0.01 ^a	0.88 \pm 0.03 ^a	0.74 \pm 0.02 ^b

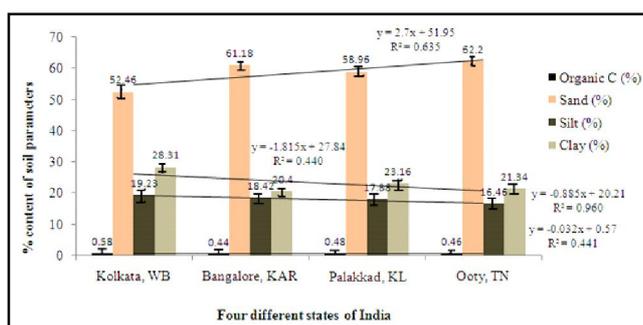


Figure 2: Geochemical properties of the collected soils

2.3 Analysis of elements in raw leaf samples

Dried plant samples are powdered and analyzed for presence of various elements, namely; Fe, Cu, Zn, Pb, Cd, Ni, As and Cr by acid digestion method using AAS. Triacid mixture was prepared by mixed with concentrated nitric acid 100 ml, concentrated sulphuric acid 10 ml and 40 ml of 60% perchloric acid. Leaf samples are pretreated sample with HNO_3 and then placed in digestion flask.

Thereafter, 2 g of powdered plant samples mixed with 6 ml of ternary acid mixture (previously prepared with three concentrated acids) and digestion was carried out at 180°C to 200°C until dense white fumes were evolved and formed residue. The residue was diluted with glass distilled water and made up to definite volume in a volumetric flask. Then the solution was ready for the estimation of Fe, Cu and Zn and toxic heavy metals like Cd, Cr, Pb, and Ni.

2.4 Collection of plant materials and processing

The leaves of MDC were collected from above mentioned four different geographic zones in the summer season (month of March-April) in 2016. Summer season was selected because during this time, the MDC plant leaves are become full matured and can thrive in environmental condition. All the leaf samples were identified and authenticated by Dr. Shivananda T.N, Principal Scientist, Department of Medicinal and Aromatic plant, Indian Institute of Horticultural Research, Bangalore, India and thereafter voucher herbarium specimens (No: MDWB-307/KCP; MDKA-308/KCP; MDTN-309/KCP and MDKL-310/KCP) was preserved in the Department of Pharmacognosy in Krupanidhi College of Pharmacy, Bangalore, India.

The leaf materials were dried in shade for 14 days after cleaning with running fresh water, then pulverized, passed through sieve no. 40 and stored in air sealed plastic cover with proper labeling and used for further investigation.

2.5 Preparation of leaf sample extract

Powdered leaves materials of MDC (250 g) was extracted separately with methanol using soxhlet method for 6-7 h. Then collected the extract and filtered with Whatman filter paper (No.1) and then evaporated to dryness at 45°C using rotary flash evaporator and percentage yield was calculated separately. Thereafter, the crude extracts were stored in screw cap small glass bottles (with proper labeling) at 4-5°C in refrigeration condition. Similarly, aqueous solvent (Distilled water) was used for leaf extraction by hot maceration method (Reflux method) for 8-9 h. Separately, extracts were filtrate with muslin cloth and evaporated to dryness by the same method as described earlier. Percentage yield was calculated and finally stored in small screw cap glass bottles with labeled properly, kept in refrigerator at 4-5°C until used further.

Preliminary phytochemical tests were performed to understand the group of secondary metabolites present in both the extracted leaf samples as per the standard method described by Harborne (1973). For the test, 1 ml of all the extracted samples was used for the test except determination of saponins (5 ml).

2.6 Determination of alkaloid content

1 mg of each plant extracts were dissolved in 2 ml of dimethyl sulphoxide (DMSO) and 2 ml of concentrated hydrochloric acid and filtered. This solution was transferred to a separating funnel and then 5 ml of bromocresol green and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by shaking and after layer separation, the down portion was collected. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) and the absorbance for test and standard solutions were determined at 470 nm against the reagent blank with an UV spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract (Mallikarjuna Rao *et al.*, 2012).

2.7 Determination of total phenolic content

Total phenolics content in all the extracts was determined using spectrophotometric Folin-Ciocalteu assay method. 1 ml of extract dissolved in 9 ml of distilled water and to that 1 ml of Folin-Ciocalteu phenol reagent was added. After 5 minutes, 10 ml of 7 % Sodium carbonate solution was mixed to the mixture. The volume was made up to 25 ml. Thereafter, standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared. Incubated for about 2 h. at room temperature and the absorbance for test and standard solutions were determined at 550 nm using UV spectrophotometer. Blank sample was prepared for reading corrections. Total phenol content was expressed as mg of gallic acid equivalent of extract (Singleton *et al.*, 1999; Ali Ghasemzadeh *et al.*, 2010).

2.8 Determination of tannin content

Total tannins were determined by Folin-Ciocalteu method using UV spectrophotometer. About 0.1 mg of the sample extract dissolved in 7.5 ml of distilled water and then 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35 % sodium carbonate solution were added and diluted to 10 ml with distilled water. The mixture solution was shaken and kept at room temperature for 45 min. Standard solutions

of gallic acid at the concentration of 20, 40, 60, 80 and 100 µg/ml were prepared and absorbance recorded at 725 nm against blank (for reading correction). The tannin content was expressed in terms of mg of gallic acid equivalent of extract (Singh *et al.*, 2012; AfifyAel *et al.*, 2012).

2.9 Anthelmintic activity

2.9.1 Test animal

Adult earthworms (*Pheretima posthuma*) were collected from moist soil of Basavangudi area of Bangalore, Karnataka and cleaned with water to remove all dirt matters. Then the worms were used for *in vitro* anthelmintic study. Earthworm was selected due to its anatomical and physiological resemblance with the human intestinal roundworm parasites. The earthworms of 6-8 cm in lengths and 0.2-0.4 cm in width were used for all the experimental protocol.

2.9.2 Assay method

Three earthworms were placed in each petridish containing 20 ml of MDC aqueous and methanol extracts at four different concentrations (50, 100, 150 and 200 mg/ml). Albendazole (25 and 50 mg/ml) was used as reference standard (dissolved in anhydrous formic acid) whereas distilled water was used as control. Thereafter, observed for time of paralysis and death of worms. The mean time for paralysis was recorded when after shaken continuously for few seconds, no movement observed and the time for death of worm (min) was recorded even after given external stimulation, there was no movement at all, *i.e.*, total loss of motility, followed by fading away of the body color of the worms. Each experiment was carried out 3 times and the results were expressed in comparison to the standard drug Albendazole.

2.10 Statistical analysis

Statistically, t-test was performed with four state samples for testing the significance differences in all the elements. Before conducting t-test, equality of variances was tested by using F-test. A brief discussion of the above statistical test procedures are given below.

2.10.1 Equality of variance test

For testing the equality of variances of four populations, the F-test statistic as given by $F = s_1^2/s_2^2, s_3^2, s_4^2$ are used where s_1^2, s_2^2, s_3^2 and s_4^2 are the sample variance of population 1, 2, 3 and 4, respectively. It is assumed that s_1^2 is the largest among four variances. The F statistic follows F distribution with $(n_1-1, n_2-1, n_3-1, n_4-1)$ degrees of freedom.

2.10.2 Hypothesis testing with four independent samples t

The assumptions for four sample t-test are either the four samples are independent or the four samples are randomly selected from normally distributed populations or the population variances are equal. Assume that, $(\mu_1, \sigma_1^2), (\mu_2, \sigma_2^2), (\mu_3, \sigma_3^2)$ and (μ_4, σ_4^2) are the population mean and variances of four populations, respectively. $(\bar{x}_1, s_1^2), (\bar{x}_2, s_2^2), (\bar{x}_3, s_3^2)$ and (\bar{x}_4, s_4^2) are the sample mean and sample variance of four samples, respectively. n_1, n_2, n_3 and n_4 are the sample size of four samples, respectively. The results were expressed as Mean \pm SE \bar{x} ($n=3$).

Thereafter, correlation coefficient between the heavy metals of each state was determined by linear correlation coefficient (Pearson method) using the following formula:

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2} \sqrt{n(\sum y^2) - (\sum y)^2}}$$

where, r = Correlation coefficient, n = no of pairs of data, x and y are data of individual pair.

Thereafter, repeated ANOVA with post “Dunnett’s t- test” was performed for total alkaloids, total phenols, total tannins content and anthelmintic assay in the different extracts and further more correlation matrix determined at confidence intervals 95% with two tailed where p value at < 0.05 was considered as statistically significant. Data were processed with Microsoft excel.

3. Results

3.1 Physical and chemical properties of soils

Selected physical and chemical properties of four experimental sites have been tabulated in Table 1. The physical and chemical properties of experimental soils were appeared to be geographic condition dependent. pH levels of MDC-WB was more acidic (pH= 6.32 ± 0.14) that of other zones. Thereafter, EC was found significantly higher in MDC-KAR (15.20±0.23) than other States (p≤0.05). There was no significant difference found in organic carbon between the soils. The soil texture of WB was found sandy loam even though CEC content of MDC-WB was higher (18.68 ± 0.31) than that of other States. Total metals content in soils was followed, the following trend Fe > Zn > Cu irrespective of location but content of Ni, Pb, Cr and Cd content varied with the soil nature. The trend of DTPA extractable metals in soils was found higher Fe content but varied Pb, Ni, Cu, Cr, Cd and Zn content with the climatic condition.

The heavy metals associated with different fractions in WB series (MDC-WB) follow the order: **Cd:** F6 (1.12 mg kg⁻¹) > F4 (0.62 mg kg⁻¹) > F5 (0.21 mg kg⁻¹) > F3 (0.10 mg kg⁻¹) > F2 (0.04 mg kg⁻¹) > F1 (0.02 mg kg⁻¹); **Cr:** F6 (1.87 mg kg⁻¹) > F4(0.76 mg kg⁻¹) > F3(0.44 mg kg⁻¹) > F2(0.11 mg kg⁻¹) > F5(0.05 mg kg⁻¹) > F1(0.01 mg kg⁻¹); **Cu:** F6 (6.54 mg kg⁻¹) > F3 (2.01 mg kg⁻¹) > F5 (1.57 mg kg⁻¹) > F4 (0.84 mg kg⁻¹) > F2 (0.32 mg kg⁻¹) > F1(0.04 mg kg⁻¹); **Fe:** F5 (14.34 mg kg⁻¹) > F6 (10.12 mg kg⁻¹) > F3 (5.04 mg kg⁻¹) > F4 (3.07 mg kg⁻¹) > F2 (1.48 mg kg⁻¹) > F1 (0.61 mg kg⁻¹); **Ni:** F6 (2.01 mg kg⁻¹) > F5 (1.07 mg kg⁻¹) > F4 (0.72 mg kg⁻¹) > F1(0.39 mg kg⁻¹) > F3 (0.10 mg kg⁻¹) > F2 (0.02 mg kg⁻¹); **Pb:** F6(1.14 mg kg⁻¹) > F4(1.03 mg kg⁻¹) > F5(0.48 mg kg⁻¹) > F3(0.13 mg kg⁻¹) > F1 (0.06 mg kg⁻¹) > F2(0.02 mg kg⁻¹); **Zn:** F6(13.12 mg kg⁻¹) > F4(9.04 mg kg⁻¹) > F3(2.07 mg kg⁻¹) > F2(1.71 mg kg⁻¹) > F1(0.28 mg kg⁻¹) > F5(0.07 mg kg⁻¹).

The heavy metals associated with different fractions in Bangalore series (MDC-KAR) follow the order:

Cd: F6 (4.18 mg kg⁻¹) > F5 (1.68 mg kg⁻¹) > F3 (1.19 mg kg⁻¹) > F2 (0.10 mg kg⁻¹) > F1 (0.04 mg kg⁻¹) > F4 (0.02 mg kg⁻¹); **Cr:** F6 (3.87 mg kg⁻¹) > F5(1.76 mg kg⁻¹) > F3(0.51 mg kg⁻¹) > F1(0.16 mg kg⁻¹) > F4(0.08 mg kg⁻¹) > F2(0.03 mg kg⁻¹); **Cu:** F5 (6.57 mg kg⁻¹) > F3 (3.03 mg kg⁻¹) > F6 (2.55 mg kg⁻¹) > F1 (0.79 mg kg⁻¹) > F2 (0.33 mg kg⁻¹) > F4(0.04 mg kg⁻¹); **Fe:** F4 (34.40 mg kg⁻¹) > F6 (22.12 mg kg⁻¹) > F5 (13.05 mg kg⁻¹) > F2 (9.07 mg kg⁻¹) > F3 (5.47 mg kg⁻¹) > F1

(2.60 mg kg⁻¹); **Ni:** F6 (3.01 mg kg⁻¹) > F5 (2.07 mg kg⁻¹) > F3 (1.94 mg kg⁻¹) > F1(0.84 mg kg⁻¹) > F2 (0.57 mg kg⁻¹) > F4 (0.38 mg kg⁻¹) ; **Pb:** F6(6.11 mg kg⁻¹) > F4(3.21 mg kg⁻¹) > F5(1.43 mg kg⁻¹) > F2(0.53 mg kg⁻¹) > F3 (0.26 mg kg⁻¹) > F1(0.02 mg kg⁻¹); **Zn:** F4(28.11 mg kg⁻¹) > F5(18.10 mg kg⁻¹) > F3(7.14 mg kg⁻¹) > F6(3.84 mg kg⁻¹) > F1(0.86 mg kg⁻¹) > F2(0.14 mg kg⁻¹).

The heavy metals associated with different fractions in Palakkad series (MDC-KL) follow the order:

Cd: F6 (1.24 mg kg⁻¹) > F5 (0.42 mg kg⁻¹) > F3 (0.37 mg kg⁻¹) > F2 (0.18 mg kg⁻¹) > F4 (0.08 mg kg⁻¹) > F1 (0.05 mg kg⁻¹); **Cr:** F6 (2.15 mg kg⁻¹) > F5(0.74 mg kg⁻¹) > F3(0.41 mg kg⁻¹) > F2(0.31 mg kg⁻¹) > F4(0.12 mg kg⁻¹) > F1(0.04 mg kg⁻¹); **Cu:** F5(6.54 mg kg⁻¹) > F3(3.14 mg kg⁻¹) > F6(1.59 mg kg⁻¹) > F2(0.98 mg kg⁻¹) > F4(0.54 mg kg⁻¹) > F1(0.16 mg kg⁻¹); **Fe:** F5 (24.34 mg kg⁻¹) > F6 (18.12 mg kg⁻¹) > F4 (12.29 mg kg⁻¹) > F3 (7.17 mg kg⁻¹) > F1 (4.81 mg kg⁻¹) > F2 (1.82 mg kg⁻¹); **Ni:** F6 (3.01 mg kg⁻¹) > F5 (1.54 mg kg⁻¹) > F2 (0.94 mg kg⁻¹) > F1(0.80 mg kg⁻¹) > F3 (0.49 mg kg⁻¹) > F4 (0.11 mg kg⁻¹); **Pb:** F6(2.14 mg kg⁻¹) > F4(1.78 mg kg⁻¹) > F5(0.84 mg kg⁻¹) > F3(0.53 mg kg⁻¹) > F2 (0.38 mg kg⁻¹) > F1(0.12 mg kg⁻¹); **Zn:** F4(10.12 mg kg⁻¹) > F5(6.04 mg kg⁻¹) > F6(2.07 mg kg⁻¹) > F2(1.73 mg kg⁻¹) > F1(0.46 mg kg⁻¹) > F3(0.13 mg kg⁻¹).

The heavy metals associated with different fractions in Ooty series (MDC-TN) follow the order:

Cd: F6 (2.10 mg kg⁻¹) > F5 (1.57 mg kg⁻¹) > F4 (0.61 mg kg⁻¹) > F1 (0.24 mg kg⁻¹) > F2 (0.10 mg kg⁻¹) > F3 (0.04 mg kg⁻¹); **Cr:** F4 (2.87 mg kg⁻¹) > F6(1.64 mg kg⁻¹) > F2(0.64 mg kg⁻¹) > F3(0.51 mg kg⁻¹) > F5(0.25 mg kg⁻¹) > F1(0.06 mg kg⁻¹); **Cu:** F6 (6.54 mg kg⁻¹) > F1(2.76 mg kg⁻¹) > F4 (1.65 mg kg⁻¹) > F5 (0.84 mg kg⁻¹) > F2 (0.36 mg kg⁻¹) > F3(0.08 mg kg⁻¹); **Fe:** F6 (28.04 mg kg⁻¹) > F4 (21.10 mg kg⁻¹) > F5 (14.04 mg kg⁻¹) > F3 (5.12 mg kg⁻¹) > F2 (3.42 mg kg⁻¹) > F1 (0.96 mg kg⁻¹); **Ni:** F6 (3.38 mg kg⁻¹) > F5 (2.62 mg kg⁻¹) > F4 (0.74 mg kg⁻¹) > F3(0.43 mg kg⁻¹) > F2 (0.22 mg kg⁻¹) > F1 (0.06 mg kg⁻¹); **Pb:** F5(6.09 mg kg⁻¹) > F4(3.00 mg kg⁻¹) > F3(1.26 mg kg⁻¹) > F6(0.13 mg kg⁻¹) > F1 (0.06 mg kg⁻¹) > F2(0.02 mg kg⁻¹); **Zn:** F6(11.02 mg kg⁻¹) > F4(7.12 mg kg⁻¹) > F5(2.14 mg kg⁻¹) > F2(1.60 mg kg⁻¹) > F3(0.38 mg kg⁻¹) > F1(0.05 mg kg⁻¹).

3.2 Heavy elements in leaf samples

The mean concentration levels of metals in methanol and water extracts of leaf are summarized in Table 2. A perusal of data in Table 2 shows that only Cu, Fe and Zn were found in leaf extracts but below detectable levels, perhaps no results were found for non-essential heavy metals like Ni, Cd, Cr and Pb for all the samples. Among the extracts, methanol extract from MDC-WB showed comparatively better when compared with elemental contents from all other samples. Correlation coefficient among the metals are tabulated that showed statistically negative correlation mostly among the Zn and Cu for all the state samples (Table 3).

3.3 Yield of the leaf extracts

The yield of the leaf extracts are presented in Figure 3. The figure described that methanol extract of MDC-WB given higher yield (22.48% w/w), followed by methanol extract of MDC-KL (20.96% w/w). Furthermore, aqueous extract of MDC-WB showed better percentage of yield (18.04 % w/w), compared to other extracts but marginal non-significant differences observed between MDC-KAR and MDC-TN extracts when compared with the geographical sources.

Table 2: Elemental analysis in leaf samples by AAS (Values represent mean of three replications \pm SEM; same letter(s) in a particular row represent non-significant difference between the samples)

Elements	Location with soil sample ID (mg kg ⁻¹)			
	Kolkata WB	Bangalore KAR	Palakkad KL	Ooty TN
Fe	5.12 \pm 0.011 ^b	4.78 \pm 0.012 ^a	5.02 \pm 0.010 ^b	4.75 \pm 0.003 ^a
Cu	0.70 \pm 0.008 ^a	0.68 \pm 0.003 ^a	0.71 \pm 0.008 ^a	0.69 \pm 0.009 ^a
Zn	0.96 \pm 0.008 ^b	0.84 \pm 0.008 ^a	0.87 \pm 0.012 ^a	0.82 \pm 0.008 ^a
Ni	BDL	BDL	BDL	BDL
Cr	BDL	BDL	BDL	BDL
Pb	BDL	BDL	BDL	BDL
Cd	BDL	BDL	BDL	BDL
F value	49140			
R ² value	1.000			

• BDL = Below detectable limit; R² = Coefficient of determination

Table 3: Correlation coefficient between all the elements of each state

HM	MDC-WB			MDC-KAR			MDC-KL			MDC-TN		
	Fe	Cu	Zn	Fe	Cu	Zn	Fe	Cu	Zn	Fe	Cu	Zn
Fe	1			1			1			1		
Cu	0.654	1		-0.693	1		-0.944	1		0.776	1	
Zn	-0.981	-0.785	1	0.419	-0.944	1	-0.970	0.995	1	-0.189	-0.786	1

MDC = *Melia dubia* Cav.

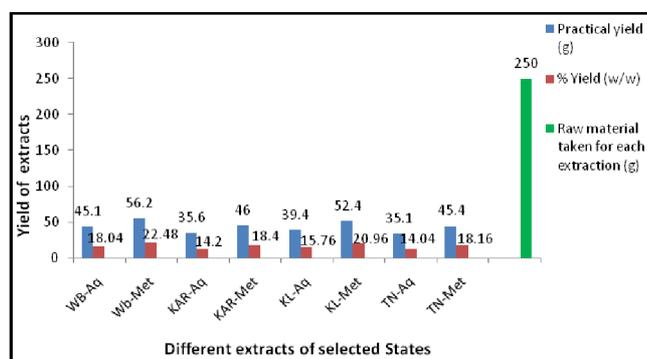


Figure 3: Yield of the extracts of MDC leaf collected from various States

Preliminary chemical tests were carried out as per the method described above and resulted the presence of alkaloids, phenols, flavonoids, steroids, tannins, carbohydrate and proteins in all the methanol extracts whereas flavonoids, glycoside, alkaloids, phenols, carbohydrates are showed in aqueous extracts in all the sources.

3.4 Total alkaloids, phenolics and tannins content

The alkaloid contents was determined for all the MDC extracts and expressed in terms of atropine equivalent as mg of AE/g of extract (the standard curve equation: $y = 0.005x - 0.057$, $R^2 = 0.996$, Figure 4). The significant highest concentration of alkaloid was measured 82.71 mg/g in methanol extract of MDC-WB, followed by MDC-KL methanol extract (78.86 mg/g). Thereafter, the same trend followed with the aqueous extracts. MDC-WB showed alkaloid content of 62.42 mg/g followed, by 58.50 mg/g

with MDC-KL aqueous extract whereas both the extracts of KAR and TN showed marginal variance of alkaloid contents that are statistically non-significant (Table 4).

Similarly, the phenolics content was determined and expressed in terms of gallic acid equivalent as mg of GAE/g of extract (the standard curve equation: $y = 0.007x - 0.062$, $R^2 = 0.994$, Figure 5). The significant highest concentration of phenolics was reported with methanol extract of MDC-WB (102.13 mg/g), followed by methanol MDC-KL extract (99.26 mg/g). Thereafter, aqueous extract of MDC-WB showed phenolics content of 100.39 mg/g, followed by 98.76 mg/g with aqueous MDC-KL extract (Table 4).

Total tannins were reported for all the extracts of different zones which were varied with different extracts when compared with standard GAE/g of extract. However, the same was significantly increased with MDC-WB methanol sample (64.24 mg/g), followed by MDC-KL methanol extract (58.36 mg/g). But non-significant data revealed for aqueous extracts of MDC-WB and MDC-KL (Table 4).

3.5 Anthelmintic activity

The anthelmintic activity of methanol and aqueous leaves extract of MDC exhibited anthelmintic activity, using *Pheretima posthuma* in dose dependent manner giving shortest time of paralysis (TTP) and death (TTD) with 150 mg/ml concentration. The MDC-WB methanol extract caused paralysis and time of death was 6.47 ± 0.12 min and 9.42 ± 0.11 min, respectively followed, by MDC-KL sample at the dose of 150 mg/ml (TTP: 8.47 ± 0.02 and TTD: 10.40 ± 0.01 min). The same trend followed in case of aqueous extracts and the activity was better in MDC-WB (TTP: 10.3 ± 0.10 and TTD: 16.27 ± 0.10 min), followed by MDC-KL extract (TTP: 11.46 ± 0.01 and TTD: 117.14 ± 0.20 min). There are not much differences observed in case of MDC-KAR and MDC-TN samples but all the values are more than that of standard albendazole. The standard at 50 mg/ml has gave very less time for TTP (6.32 ± 0.01) and TTD (10.48 ± 0.12). From the above results, extracts of MDC leaves produced a significant ($P < 0.01$) anthelmintic activity in dose dependent manner as shown in Table 5 and Figure 6. Thereafter, correlation coefficient between all the extracts of different States was analyzed for TTP and TTD (Tables 6 and 7) and resulted significant correlation varied with the extracts among the four different States. This indicates the activity mainly depends on the solvents used for the extraction.

4. Discussion

4.1 Physical and chemical properties of soils

pH of the soil indicate that experimental soils in the study areas were slightly basic to neutral soils. High EC values may reflect the considerable amount of soluble salts in soils, and high EC values in soils may resist the growth of medicinal plant and agricultural crops (Chai *et al.*, 2015) and, thereafter relatively low organic carbon in experimental soils suggested that the soils are not fertile under the favorable environmental conditions (Musunguzi *et al.*, 2015) and that may be the result where MDC-KAR has not given satisfactory yield. The lower CEC of KAR as compare to other States could be due to presence of lower amount of humidified carbon that decreased the formation of carboxyl and phenolic functional groups, which in turn, contributed to lower CEC (Karak *et al.*, 2014). Literature survey resulted that heavy metals are necessary for several physiological functions in trace amount for plant and other

organism, but their elevated concentrations could have adverse effect (Kabata-Pendias and Pendias, 2011). Furthermore, metals in experimental soils are the sources of parent materials from which the soils developed and anthropogenic sources includes atmospheric deposition, road side runoff and industrial waste discharges (Karak *et al.*, 2013). In our report, it was observed that Fe, Cu and Zn

content was more with increased with soil acidity whereas DTPA extractable Cd, Cr, Cu, Fe, Ni, Pb and Zn in soils contributed relatively optimum where soil pH is between 6 to 6.5. Furthermore, the small ratios of DTPA extractable metals with respect to total metal can't identify the role of climatic conditions, soil genesis and geological history in those extractable metals in soils.

Table 4: Alkaloid, tannins and phenol contents in different extract (same letters are statistically non significant between the States, n = 3)

	WB-Met	WB-Aq	KAR-Met	KAR-Aq	KL-Met	KL-Aq	TN-Met	TN-Aq
Alkaloids	82.71 ^{**} ±0.12	62.42 ^{**} ±0.20	72.21 ^{a**} ±0.11	56.84 ^{b**} ±0.02	78.86 ^{**} ±0.01	58.50 ^{**} ±0.22	71.36 ^{a**} ±0.12	56.72 ^{b**} ±0.11
Source of variation			Degree of freedom			Mean square		
Treatments (Between columns)			7			316.24		
Residuals (within columns)			16			0.030		
F			-			10541.33		
Total Phenolics	102.13 ^{**} ±0.01	100.39 ^{**} ±0.02	98.03 ^{a**} ±0.22	96.53 ^{**} ±0.02	99.26 ^{b**} ±0.01	98.76 ^{b,c**} ±0.01	98.43 ^{b,c**} ±0.22	95.87 ^{**} ±0.02
Source of variation			Degree of freedom			Mean square		
Treatments (Between columns)			7			12.074		
Residuals (within columns)			16			0.0369		
F			-			327.21		
Tannins	64.24 ^{**} ±0.13	62.56 ^{a**} ±0.21	57.76 ^{b**} ±0.11	55.43 ^{**} ±0.12	58.36 ^{b**} ±0.01	62.12 ^{a**} ±0.01	56.76 ^{c**} ±0.11	56.32 ^{c**} ±0.21
Source of variation			Degree of freedom			Mean square		
Treatments (Between columns)			7			32.767		
Residuals (within columns)			16			0.053		
F			-			618.25		

**p < 0.05= Significant; Met = Methanol extract; Aq = Aqueous extract

Table 5: Anthelmintic activity of plant extracts against *Pheretima posthuma*

Group	Conc(mg/ml)	MDC-WB		MDC-KAR		MDC-KL		MDC-TN	
		TTP(Min)	TTD(Min)	TTP(Min)	TTD(Min)	TTP(Min)	TTD(Min)	TTP(Min)	TTD(Min)
Control	-	-	-	-	-	-	-	-	-
Albendazole	25	9.34 ±0.11	11.21±0.12	9.34 ±0.11	11.21±0.12	9.34 ±0.11	11.21±0.12	9.34 ±0.11	11.21±0.12
	50	6.32± 0.01	10.48±0.12	6.32±0.01	10.48±0.12	6.32±0.01	10.48±0.12	6.32 ±0.01	10.48±0.12
Met extract	25	14.2 [*] ±0.02	19.43 [*] ±0.02	15.03 [*] ±0.11	19.54 [*] ±0.12	14.5 [*] ±0.12	19.53 [*] ±0.12	15.13 [*] ±0.11	19.48 [*] ±0.22
	50	9.57 [*] ±0.21	15.32 [*] ±0.03	10.32 [*] ±0.22	16.32 [*] ±0.11	10.12 [*] ±0.11	15.52 [*] ±0.01	10.47 [*] ±0.01	16.33 [*] ±0.12
	100	7.21 [*] ±0.02	12.4 [*] ±0.10	9.46 [*] ±0.01	14.04 [*] ±0.12	9.41 [*] ±0.02	13.56 [*] ±0.10	10.26 [*] ±0.22	14.12 [*] ±0.11
Aqu extract	150	6.47 [*] ±0.12	9.42 [*] ±0.11	9.02 [*] ±0.12	10.52 [*] ±0.01	8.47 [*] ±0.02	10.40 [*] ±0.01	9.14 [*] ±0.12	10.43 [*] ±0.10
	25	16.30 [*] ±0.12	22.10 [*] ±0.14	17.40 [*] ±0.10	23.14 [*] ±0.10	17.25 [*] ±0.02	22.38 [*] ±0.10	17.54 [*] ±0.01	23.31 [*] ±0.11
	50	13.53 [*] ±0.02	20.03 [*] ±0.11	15.42 [*] ±0.12	22.46 [*] ±0.22	15.30 [*] ±0.01	21.31 ^{a*} ±0.10	15.55 [*] ±0.22	22.40 [*] ±0.01
	100	11.32 [*] ±0.10	18.41 [*] ±0.02	14.03 [*] ±0.01	20.46 [*] ±0.11	13.21 [*] ±0.11	19.34 [*] ±0.02	14.56 [*] ±0.12	20.32 [*] ±0.01
	150	10.3 [*] ±0.10	16.27 [*] ±0.10	12.02 [*] ±0.11	17.54 [*] ±0.22	11.46 [*] ±0.01	17.14 [*] ±0.20	12.47 [*] ±0.11	17.38 [*] ±0.12

* p < 0.001 = Significant when compared with the control, values are calculated by using ONE-way-ANOVA followed by Dunnett's t test; TTP = Time taken for paralysis; TTD = Time taken for death; MDC = *Melia dubia* Cav.

Table 6: Correlation coefficient between extracts of all the States against *P. posthuma* paralysis time

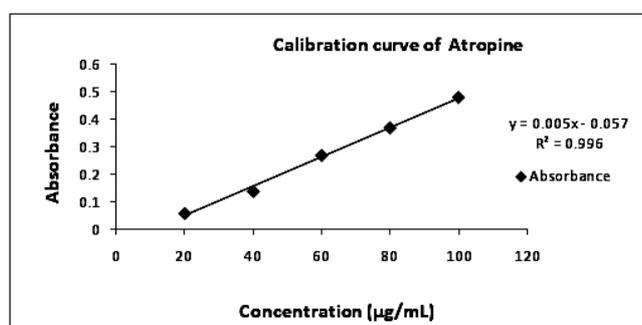
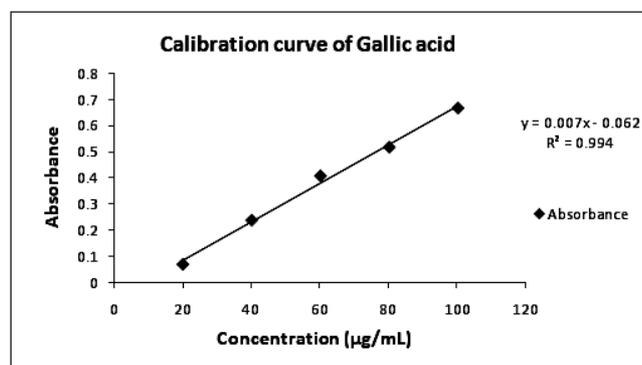
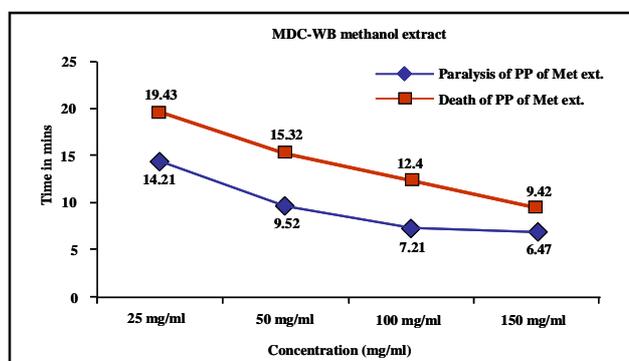
	WB-Met (TTP)	KAR-Met (TTP)	KL-Met (TTP)	TN-Met (TTP)	WB-Aqu (TTP)	KAR-Aqu (TTP)	KL-Aqu (TTP)	TN-Aqu (TTP)
WB-Met (TTP)	1							
KAR-Met (TTP)	0.981	1						
KL-Met (TTP)	0.984	0.996*	1					
TN-Met (TTP)	0.968	0.993	0.997*	1				
WB-Aqu (TTP)	0.989	0.944	0.956	0.933	1			
KAR-Aqu (TTP)	0.944	0.888	0.918	0.899	0.976	1		
KL-Aqu (TTP)	0.952	0.886	0.912	0.887	0.985	0.995*	1	
TN-Aqu (TTP)	0.937	0.888	0.920	0.905	0.966	0.998**	0.987	1

Significant at *p =0.05; TTP = Time taken for paralysis

Table 7: Correlation coefficient between extracts of all the States against *P. posthuma* death time

	WB-Met (TTD)	KAR-Met (TTD)	KL-Met (TTD)	TN-Met (TTD)	WB-Aqu (TTD)	KAR-Aqu (TTD)	KL-Aqu (TTD)	TN-Aqu (TTD)
WB-Met (TTD)	1							
KAR-Met (TTD)	0.994	1						
KL-Met (TTD)	0.997*	0.995	1					
TN-Met (TTD)	0.992	0.999**	0.994	1				
WB-Aqu (TTD)	0.996*	0.999**	0.995	0.998**	1			
KAR-Aqu (TTD)	0.942	0.967	0.939	0.970	0.965	1		
KL-Aqu (TTD)	0.973	0.986	0.968	0.987	0.987	0.992	1	
TN-Aqu (TTD)	0.953	0.975	0.950	0.978	0.974	0.999**	0.996*	1

Significant at *p =0.05; TTD = Time taken for death

**Figure 4:** Standard curve for atropine at UV 470 nm**Figure 5:** Standard curve for gallic acid at UV 550 nm**Figure 6:** Anthelmintic activity methanol extract of MDC-WB
PP = *Pheretima posthuma*

4.2 Metal content in leaf samples

All the leaf samples were estimated for the content of metals in raw leaf samples estimated by AAS and found higher in MDC-WB sample, followed by MDC-KL sample. This may be due to the presence of micronutrients in the soil and due to the optimum soil pH. Gregor (2004) reported several abiotic factors influence the availability of metal to plants, including pH, temperature, redox potential, cation exchange capacity and organic matter. Similarly optimum soil pH enhanced the uptake of Fe, Cu and Zn by the plant which already revealed by earlier (Das and Tribedi, 2015) and this investigation followed the same trend. Earlier literatures supported that calcareous soil content less metal ions to available for plant uptake, *i.e.*, essential micronutrients (Aref, 2012; Das, 2014).

4.3 Yield of extract

The percentage yield of the extract was differs with the sample collected from four different States. Interestingly, the higher yield obtained for MDC-WB, followed by MDC-KL. Many literatures revealed that solvents and time of extraction affect the percentage yield of the extract (Sultana *et al.*, 2009; Anwar *et al.*, 2013), not only that yield of extract also directly correlated with the leaf biomass and choice of solvent (Das *et al.*, 2012; Dent *et al.*, 2013). In correlation, the higher yield also may be due to the soil nature and the content of the metals present in the soil. The enhanced content of soil Fe and Zn increase the leaf biomass which was reported by Kumar *et al.* (2009). Our investigation also followed the same trend and based on that methanol extract showed good percentage of extract for all the samples collected from different States.

4.4 Total alkaloids, phenolics and tannins content

Total alkaloid, phenolics and tannins content were determined for all the extracts and revealed higher amount in methanol extract for all the States. It was important to determine alkaloids, phenolics and tannins because these constituents are the main responsible for anthelmintic activity (Acharya *et al.*, 2011; Dash *et al.*, 2010) Methanol extract of MDC-WB showed maximum content of alkaloids, phenolics and tannins, followed by methanol extract of MDC-KL. This may be due to the presence of metal ions in the leaf that may be enhances the biochemical process and resulted increase amount of secondary metabolites. These results may be due to the interaction among the environmental conditions because low temperature with high acidic soil nature may increase production of total phenolics by enhancing the synthesis of phenylalanine ammonia lyase in plants (Kishore *et al.*, 2010). Furthermore, the recovery of phenolic content in plant leaf samples are influenced by the polarity of extracting solvents and solubility of the compound in the solvent used for extraction (Sulaiman *et al.*, 2011) that same principle followed in the present study where methanolic extract showed significant results. It was reported also that the biological effects of metals (Zn, Fe, Cu, Mn, Ca, Mg, Ni, Co, K and Na) in living systems strongly depend on their concentration (Ennever, 1994). Based on that, Fe, Cu and Zn concentration was higher in MDC-WB leaf sample, followed by MDC-KL and the similar results obtained as stated above.

4.5 Anthelmintic activity

In this present study, albendazole was used as standard drug because it increases chloride ion conductivity of worm muscle membrane and produces hyper polarization and reduced excitability. This mechanism led to muscle relaxation and flaccid paralysis (Mali *et al.*, 2005). Thereafter, methanol and aqueous leaf extracts of MDC revealed significant paralysis activity and even death of the earthworms (*Pheretima posthuma*) at concentration dependent manner in shorter time. This activity may be due to the presence of various phytoconstituents in the leaf extract of MDC. Tannins and phenolics are known to interfere with the energy generation in parasites by uncoupled oxidative phosphorylation (Athnasiadou *et al.*, 2001) and bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite that leads to death. Furthermore, estimation of alkaloids, tannins and phenolics in MDC was resulted high content in methanol extract that supported strong anthelmintic activity which was similar as per the earlier research evident (Kosalge and Fursule, 2009; Nayak *et al.*, 2012).

5. Conclusion

In the present study, MDC leaf sample and soil sample were collected from four different States of India and analyzed for various metal contents in the soil as well as in the raw leaf sample using AAS. Data analysis revealed MDC-WB sample gave overall satisfactory significant result. Thereafter, all the extract showed promising anthelmintic activity against earthworms (*Pheretima posthuma*) but in both the methanol and aqueous extracts, methanol extract from WB sample gave better result, followed by KL methanol sample but the activity was concentration dependent. Furthermore, overall investigation correlated the anthelmintic activity not only depends on solvent effects but also depends on the metal ion content and phytoconstituents content in the leaf sample.

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Conflict of interest

We declare that we have no conflict of interest.

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