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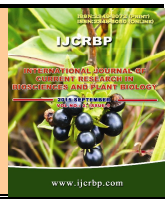

Original Research Article

Phytochemical Screening of Leaves, Bark and Wood of *Tamarindus indica* Linn. Subjected to Cement Dust Pollution

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Abstract

An investigation of phytochemical screening was conducted to study the effect of cement dust pollution on leaves, bark and wood of *Tamarindus indica* and its effect was analyzed by using various solvents which included methanol, ethanol, chloroform, ethyl acetate and benzene. *Tamarindus indica* has been of keen interest is due to its excellent medicinal values. This investigation has been done on above plant grown in two different locations one served as control and the other in the cement dust polluted site. The results of the phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, phenols, carbohydrates, proteins, amino acids, steroids and tannins except sterols and anthraquinine in control leaves, bark and wood whereas cardiac glycosides, terpenoids, sterols and anthraquinone were absent in polluted leaves, bark and wood.

Keywords

Cement dust pollution

Phytochemical screening

Tamarindus indica

Introduction

India ranks as the second largest cement producing country in the world. There are about 158 large cement plants in India. In industrial sector, cement industry is the second largest emitter of carbon dioxide and accounts for 5 per cent of global manmade carbon dioxide emissions, of which 60 per cent is from the chemical process and 40 per cent from burning fuel. Utilization of fly ash in cement sector is therefore very low. The main sources of fugitive emission in cement industry are open air handling and storage of raw materials and clinker; transfer; points; leaking joints; loading and unloading operation and vehicular movement on unpaved roads. Rajasthan, Gujarat and Tamil Nadu have 16, 12 and 10 plants each and they collectively contribute 35.79 million tons of cement (Dubey, 2013).

The biological activities of particles at various locations necessarily vary because of differing pollutant source profiles. These variations are expressions of both quantitative and qualitative differences in the relative amount of sulfuric acid mist, sulfates, or other reactive substances in the particulate mix or the relative amounts of specific carcinogenic compounds in the organic fraction of airborne particulate. The dust capture by plant is a unique combination of concentration and exposure period to the pollutant (or pollutants), of plant species, plant age and of environmental conditions

(Central pollution control board, 2007). Cement dust is one of the most important pollutants in the environment which poses a threat to the proper functioning of plant in the vicinity of cement factories (Singh, 1980) and this is adversely affecting human health.

One of the most critical impacts of cement manufacturing is the dust generated during transport, storage, milling, packing etc. Atmospheric dust is an important source of air pollution particularly in dry climates. (Dubey, 2013). The main impacts of the cement activity on the environment are the broadcasts of dusts and gases. These particles or dusts are very numerous and varied. This diversity is assigned to the different sources of broadcast (Laj and Sellegri, 2003). Especially, the cement dust can be emitted at every stage of cement production caused photosynthetic process, leaf stomata, discoloration, enzymatic malfunction, growth reduction and productivity of plants (Dubey,2013).

Since prehistoric times, plants have been used as a source of treatment for diseases. Medicinal plants have always proved to be the source of bioactive therapeutic phytochemicals based on their traditional belief and medicinal properties. Several plants or their parts have been exploited for medical purpose.

Today, it is a need to conserve plants for human welfare in all aspects. The demand for herbal medicines has got increased due to their natural origin and lesser side effects. The medicinal value of plants is attributed to the presence of some chemical substances. They also help in natural defense mechanism of plants. *Tamarindus indica* (Fabaceae) is a traditionally believed herbal plant used against numerous infectious diseases. The semi-deciduous tree bears alternate, paripinnate leaves that are unpalatable to cattle (Le Houerou, 1979) especially in the early dry season when many other trees have already dropped their leaves (Fandohan, 2008). The hardwood of tamarind serves to make agricultural tools and kitchen equipment (Fandohan, 2007). The tamarind tree produces edible, pod-like fruit which is used extensively in cuisines around the world. Other uses include traditional medicine and metal polish. The wood can be used in carpentry. Because of the tamarind's many uses, cultivation has spread around the world in tropical and subtropical zones.

In the present investigation, preliminary phytochemical investigation has been comparatively evaluating the effect of the cement dust pollution on leaves, wood and bark of *Tamarindus indica* with various solvents.

Description of the plant

The *Tamarindus indica* is a long-lived, medium-growth, bushy tree, which attains a maximum crown height of 12 to 18 metres (40 to 60 feet). The crown has an irregular, vase-shaped outline of dense foliage. The tree grows well in full sun in clay, loam, sandy, and acidic soil types, with a high resistance to drought and aerosol salt (wind-borne salt as found in coastal areas). Leaves are evergreen, bright green in color, elliptical ovular, arrangement is alternate, of the pinnately compound type, with pinnate venation and less than 5 cm (2 inches) in length. The branches droop from a single, central trunk as the tree matures and is often pruned in human agriculture to optimize tree density and ease of fruit harvest. At night, the leaflets close up. The tamarind does flower, though inconspicuously, with red and yellow elongated flowers. Flowers are 2.5 cm wide (one inch), five-petalled, borne in small racemes, and yellow with orange or red streaks. Buds are pink as the four sepals are pink and are lost when the flower blooms.

Materials and methods

Collection of plant materials

Plant materials were collected from the wild surrounding of India cement Limited, Sankari, Salem district. Plants were identified and authenticated at the Department of Botany, Jamal Mohamed College, Tiruchirappalli, Tamilnadu, India. Plant materials which were collected near the industry were considered as polluted and it is located around 1 km from the cement factory and the plant materials which were collected 10 km away from industry were considered as control.

Phytochemical screening for major constituents was undertaken using standard qualitative methods. The preliminary phytochemical tests were performed for testing different chemical groups present in leaf, wood and bark extracts.

Extraction procedure for powder samples

The dried leaf, wood and bark powder samples (150 gm) were extracted with methanol solvent (350 ml) for 3 days using Soxhlet extractor until complete extraction. After extraction, the sample was filtered with filter paper

(Whatman 1). The methanol solvent was evaporated using a rotary evaporator under pressure for 30 min resulting in semi solid crude extract (9.31 g). The dry methanol crude extract (0.34 g) was transferred into test tube for phytochemical screening. The methanol crude extract (9.0 g) was suspended in water (100 ml) and shaken until the crude extract dissolved. The solution was transferred into a separating funnel and extracted successively and separately.

After extraction all crude extracts were put inside the fume hood for the solvents to evaporate. After the solvent was completely evaporated the ethanol crude extracts (0.22 g), chloroform crude extracts (0.12 g), ethyl acetate crude extracts (0.15 g) benzene crude extracts (0.36 g) and residual methanol fractions (0.44 g) were obtained.

Preliminary phytochemical screening

The stock solution was prepared from each of the crude extracts such as ethanol, chloroform, ethyl acetate, benzene and methanol extracts (100 mg); and was dissolved in 10 ml of its own mother solvents. The obtained stock solutions were subjected to preliminary phytochemical screening (Harborne, 1998; Kokate, 2001).

Test for alkaloids

Dragendorff's test: One ml of the extract was added with 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test: One ml of the extract was added with 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Test for sterols (Divya, 2013)

Liebermann-Burchard test: Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

Test for phenols (Sofowara (1993) and Harborne (1973).

Two ml extract were taken into water and warmed at 45-50 0 C. Then 2 ml of 3% FeCl3 was added. Formation of green or blue colour will indicate the presence of phenols.

Test for glycosides (Sofowara (1993) and Harborne (1973).

The extract (100 mg) was dissolved in 1 ml glacial acetic acid containing 1 drop of 3% FeCl3. Then it was added with 1 ml of concentrated sulphuric acid. The formation of brown ring at the interface indicates the presence of glycosides.

Test for reducing sugars (Harbone, 1998; Kokate, 2001)

Fehling's test: One ml of the extract was added with equal quantities of Fehlings solution A and B and upon heating, formation of a brick red precipitate indicates the presence of sugars.

Test for amino acids

Ninhydrin test: Three ml of test solution was added with 3 drops of 5% ninhydrin solution in a test tube and heated in boiling water bath for 10 minutes. Formation of purple or bluish color indicates the presence of amino acids.

Test for flavonoids

Alkaline reagent test: Few drops of dilute ammonia were added to a portion of the extract and concentrated HCl was also added. A yellow colouration indicated the presence of flavonoids.

Zinc hydrochloride test: Few drops of extract were added with zinc dust and concentrated HCL. The presence of red colouration indicates the presence of flavonoids.

Test for phlobatannins

The extract of plant sample was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate indicates the presence of phlobatannins.

Test for cardiac glycosides

Keller-Killiani test: 0.5 g of extract was diluted with 5 ml water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under laid with 1ml of concentrated sulphuric acid. A

brown ring at the interface indicated the presence of a deoxysugar, characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer.

Terpenoids (Sofowara, 1993; Harborne, 1973)

Salkowski test: 0.2 g of the extract of the whole plant sample was mixed with 2ml of chloroform (CHCl) and concentrated H S0 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Test for steroids

The powder samples of *Tamarindus indica* (1 g) were dissolved in various solvents (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The colour of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for proteins

Xanthoprotein test: One ml of the extract was added with 1ml of concentrated nitric acid. A white precipitate is formed, and it is boiled and cooled. To that 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.

Test for tannins

Lead acetate test: A little quantity of the test solution was mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

Test for anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia and observed for the formation of pink or deep red colouration of aqueous layer.

Test for carbohydrates

Extract was dissolved in 5 ml of distilled water and filtered. The filtrate was divided into 2 portions and was used to test for the presence of carbohydrates using the following reagents.

Molisch's test: The filtrate was treated with 2 drops of alcoholic α- naphthol solution in a test tube. The formation of the violet ring at the junction indicates the presence of carbohydrates.

Fehling's test: Filtrate was hydrolysed with dilute HCl, and then neutralized with alkali and warmed with Fehling's A & B solutions. The formation of red precipitate indicates the presence of reducing sugars.

Results and discussion

The chemical analysis revealed the presence of medicinally active constituents. Over all fifteen bioactive constituents were tested and shown fourteen positive results. The samples were subjected to screen the active phytoconstituents with various solvents indicated the absent and present with strongly present, moderate and mild. The control and polluted leaf, wood and bark of *Tamarindus indica* have been treated with various solvents such as methanol, ethanol, chloroform, ethyl acetate and benzene. Comparatively control samples have been given noted responses than polluted samples. Leaf and bark samples were better than wood sample in both. Table 1 shows the results of the phytochemical screening of the control leaf of *Tamarindus indica.*

The result showed the presence of more flavonoids, moderate amount of alkaloids, phenols, glycosides, reducing sugars, amino acids, tannins and carbohydrates. Phlobatannins, cardiac glycosides, terpenoids, steroids and proteins in control leaves. Sterols and anthraquinones were absent. In polluted leaves, except for cardiac glycosides, terpenoids and anthraquinones, all other substances are present.

Table 2 shows the results of phytochemical screening of the control and cement dust polluted wood of *Tamarindus indica*. It indicates the presence of alkaloids, sterols, phenols, glycosides, reducing sugars, and amino acids, flavonoids, phlobatannins, steroids, proteins, tannins and carbohydrates. Reducing sugars, flavonoids, phenols and carbohydrates were present at moderate level in control wood. Proteins and anthraquinones are absent. In polluted wood, cardiac glycosides, terpenoids, proteins, tannins and anthraquinones are absent.

Table 1. Qualitative estimation of phytochemical constituents of control and cement dust polluted leaves of *Tamarindus indica* with various solvents.

	Control					Polluted				
Constituents	Methanol	Ethanol	Chloroform	Ethyl acetate	Benzene	Methanol	Ethanol	Chloroform	Ethyl acetate	Benzene
Alkaloids	++	+	-	-	-	++	+	-	-	-
Sterols	-	-	+	-	+	-	-	+	-	+
Phenols	++	+	-	-	-	+	+	-	-	-
Glycosides	++	+	-	-	-	+	-	-	-	-
Reducing sugars	++	+	-	+	-	+	+	-	-	-
Amino acids	++	+	-	+	-	+	+	-	-	-
Flavonoids	+++	+	-	-	-	+	-	-	-	-
Phlobatannins	+	-	-	-	-	+	-	-	-	-
Cardiac glycosides	+	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	-	-	-	-	-	-	-	-
Steroids	+	-	+	-	-	+	-	-	-	-
Proteins	+	+	+	+	+	+	+	+	-	+
Tannins	++	+	+	-	-	+	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-	-
Carbohydrates	++	+	++	+	++	+	+	+	-	+
+++ Strongly present, ++ Moderate, + Mild, - Negative.										

Table 2. Qualitative estimation of phytochemical constituents of control and cement dust polluted wood of *Tamarindus indica* with various solvents.

Constituents	Control					Polluted				
	Methanol	Ethanol	Chloroform	Ethyl acetate	Benzene	Methanol	Ethanol	Chloroform	Ethyl acetate	Benzene
Alkaloids	+	-	-	-	-	+	-	-	-	-
Sterols	-	-	+	-	+	-	-	+	-	+
Phenols	+	++	-	-	-	+	+	-	-	-
Glycosides	+	+	-	-	-	+	-	-	-	-
Reducing sugars	++	+	-	+	-	+	+	-	-	-
Amino acids	+	+	-	+	-	+	-	-	-	-
Flavonoids	++	+	-	-	-	+	-	-	-	-
Phlobatannin	+	-	-	-	-	+	-	-	-	-
Cardiac glycosides	+	+	-	-	-	-	-	-	-	-
Terpenoids	+	+	-	-	-	-	-	-	-	-
Steroids	+	-	+	-	-	+	-	-	-	-
Proteins	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	-	-	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-	-
Carbohydrates	++	+	+	+	+	++	+	+	-	+
++ Moderate, + Mild, - Negative.										

Various phytoconstituents such as alkaloids, phenols, glycosides, reducing sugars, amino acids, flavonoids, phlobatannins, cardiac glycosides, terpenoids, steroids, tannins, and carbohydrates were found to be present in control bark of *Tamarindus indica* and is represented in Table 3.

Table 3. Qualitative estimation of phytochemical constituents of control and cement dust polluted bark of *Tamarindus indica* with various solvents.

	Control					Polluted				
Constituents	Methanol	Ethanol	Chloroform	Ethyl acetate	Benzene	Methanol	Ethanol	Chloroform	Ethyl acetate	Benzene
Alkaloids	+	-	-	-	-	+	-	-	-	-
Sterols	-	-	+	-	+	-	-	+	-	+
Phenols	+	++	-	-	-	+	+	-	-	-
Glycosides	+	+	-	-	-	+	-	-	-	-
Reducing sugars	+	+	-	+	-	+	+	-	-	-
Amino acids	+	+	-	+	-	+	+	-	-	-
Flavonoids	++	+	-	-	-	+	-	-	-	-
Phlobatannins	+	-	-	-	-	+	-	-	-	-
Cardiac glycosides	+	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	-	-	-	-	-	-	-	-
Steroids	+	-	+	-	-	+	-	-	-	-
Proteins	+	-	+	+	+	+	+	+	-	+
Tannins	+	+	+	-	-	+	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	+	+	-	+

++ Moderate, + Mild, - Negative.

Among the control and polluted samples control leaf and bark methanol extract revealed the maximum number of phytoconstituents and ethanol, chloroform, benzene and ethyl acetate revealed in ascending. Ethyl acetate showed the low performance in screening tests. Carbohydrate present in all samples with all solvents except all polluted ethyl acetate samples.

Carbohydrates were being the leading and prominent phytoconstituent among all. Protein, reducing sugar, aminoacids, sterols, phenols, tannins, glycosides, flavonoids, steroids, alkaloids, terpenoids, phlobatannins and cardiac glycosides registered the presence next to carbohydrates. None of the solvent affords the positive response on anthraquinone. Sterols afford its presence only on chloroform and benzene.

Flavonoids were found in it's strongly presence in control methanol extract and it showed the presence of all components except the sterols and anthraquinone. Flavonoids were also useful for sexual stimulation (Benson et al., 2008), had potent anticancer activity and inhibited tumor growth (Sharma et al., 2007), inflammatory allergies, free radical scavenging, ulcers, microbes, hepatoxins, platelets aggregation, viruses and tumors (Okwu and Omodamiro, 2005).

Ethanolic extract affords nine constituents in control wood and three constituents in polluted wood, ten and six components present in control and polluted leaf samples and eight and five components present in control and polluted bark samples. Phenol is the leading component in control wood and control bark ethanol samples. Comparatively control samples showed maximum no. of constituents with moderate and mild presence.

Sterols, steroids, proteins, tannins and carbohydrates were found to be present in leaf and bark control samples, sterols, steroids and carbohydrates present in control wood sample with chloroform solvent. Steroids, proteins and carbohydrate were present in polluted leaf and bark sample whereas steroids and carbohydrates present in polluted wood sample. Steroids present only in all control samples and tannins present only in control leaf and bark samples. Terpenoids present only in control leaf, wood and bark samples expressed by methanol and ethanol extracts.

In ethyl acetate extract, reducing sugars, amino acids and carbohydrates were found to be present in leaf, wood and bark samples, whereas proteins and tannins present in leaf and bark samples. Indeed, both the bark and the leaves of *Tamarindus indica* are known to contain tannins (De Caluwe et al., 2009). Reducing sugars, amino acids present only in leaf, wood and bark samples, tannins in leaf sample, proteins in bark sample and carbohydrate present only in control samples. Negative performance was given by ethyl acetate in all polluted samples.

In benzene extract, sterols and carbohydrates were found to be found in all control and polluted leaf, wood and bark samples. Whereas, proteins afford its presence in leaf and bark samples.

Solvents from all the plant parts possessed a significant amount of phytochemicals. The presence of phytochemicals like alkaloids, tannins, amino acids, etc. in these plant parts might be responsible for their curative effects. The plant species with deep channels on leaves, dense hair of leaf surface had greater affect on dust removal while plant species with smoother leaves have weaker effect (Central Pollution Control Board, 2007). It has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides and alkaloids. These phytoconstituents may be responsible for the many pharmacological actions of the plant like wound healing (Sharmila, 2007) cholesterol lowering (Delanty and Dichter, 2000) and antidiabetic activity.

Researchers have shown that plants (including trees) can act as biological filters, removing large quantities of particles from the urban atmosphere (Central pollution control board, 2007). But, Abdel-Rahman (2012) concluded that cement dust seems to cause substantial changes to leaf physiology by destroying the photosynthetic pigments and interrupted the metabolism of carbohydrates, amino acids and proteins, also affected on the uptake and accumulation of nutrient elements from the soil which finally affected on the economic yield of fig trees (edible parts) quantitatively and qualitatively.

Seufert (1990) reported that, higher concentrations of air pollutants may increase or decrease the uptake of nutrients by the roots. According to Gupta (2012), plants growing under polluted environment show significant changes in their morphology, anatomy, physiology and biochemistry. The adverse effects of cement pollution on ecosystem have been proved so far. It is well known that different plant species vary considerably in their susceptibility to cement pollutants.

Conclusion

On the basis of this study, it could be concluded that, the cement pollution can be considerably reduces the biological activity of plants. Comparatively control plant showed the presence of various phytoconstituents with variable activities than polluted plants. Leaf is more sensitive than bark and wood. Its might be due to the deposition of dust particles on photosynthetic part of plants. Even though plant having the natural filter, it suffers from atmospheric pollution and the activity of some solvents which is used in this study might be suppressed by external factors. It shows the threat of medicinal plants in the emerging population; it insists us for further study to evaluate the chemical components.

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