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Thin Layer Chromatographic Studies and Phytochemical Analysis of Extract of *Euphorbia neriifolia* Linn. Plant

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A B S T R A C T

Euphorbia neriifolia Linn, a medicinal plant of the Euphorbiaceae family, is very rich in medicinal properties. This paper on *Euphorbia neriifolia* deals with the Qualitative analysis and Thin Layer Chromatography (TLC) of the extract of *Euphorbia neriifolia* Linn. The valuable data that was collected during research shows the presence of various phytochemicals like carbohydrates, flavonoids, saponins, alkaloids, diterpenes, and tannins in plant extracts. Various bioactive compounds are present in medicinal plants that have very important medicinal properties for the health of individuals and communities. These bioactive compounds from plants produce a definite normal biological action on the human body. TLC was carried out using a different solvent system for its polarity. TLC profiling of this plant was carried out using different concentrations of ethyl acetate extracts and showed different R_f values. The results obtained in the present investigation indicate that *Euphorbia neriifolia* is a rich source of various secondary metabolites. This can be further investigated by the isolation and identification of active biological compounds of medicinal utility in a cost-effective way. Thin layer chromatography studies of *Euphorbia neriifolia* extract constituted different coloured phytochemical compounds with different R_f values.

***Key words*:** *Euphorbia neriifolia*, Phytochemical analysis, Retention factor, TLC studies

India has the oldest and most diverse cultural tradition of the use of medicinal plants. Medicinal plants play a very crucial role in human life as well as all living organisms. These plants have all the required properties that are used by living organisms for their livelihood and health purposes [1]. In prehistoric times, our ancestors used these plants as medicine, as there was no advanced medical science. They research, study, and collect knowledge about plants and pass it on to the next generation. The new generation continues this tradition by doing research on plants to find medicine for disease. In 2003, according to a study by the World Health Organization (WHO), more than 75% of the total population of developing countries was not able to afford advanced allopathic medicine, so they preferred plant-based medicines to treat their primary health care needs [2]. The medical science of India has an official declared list of more than 45,000 plant species and more than 7000 species of medicinal plants growing in its 16 agro-favoured zones covering nearly 64 million hectares of forest land [3].

Ethno pharmacology is the diverse study of how people get medicine from shrubs, plants, water, fungi, animals, or other remaining natural resources [4]. In the current scenario, the field has mainly focused on improving new drugs on the basis of the medicinal plants used by native peoples. The revelation that indigenous information about remedial plants may hold clues for relieving diseases has ended up being one of the most extensively used conflicts for preserving society and an organic, decent variety [5]. Scientists and researchers have developed many bioactive compounds that increase the effectiveness of herbal medicine. These compounds are known as secondary metabolites of plants. Pharmacological research and testing of sources and modifying these naturally occurring sources represents a major strategy for discovering and improving new drugs [6]. Moreover, secondary metabolites of plants contain chemical and pharmaceutical properties that are most helpful for human health [7]. The secondary metabolites or bioactive compounds that are used to treat many diseases are alkaloids, cardiac glycosides, saponins, flavonoids, phenol, and steroids [8] and some of these metabolites have proved to be efficient in hampering and inhibiting various types of cancer [9]. People from every corner of the world totally depend on plants or shrubs for habitat as they have their own minds about medicinal properties and value. Most diseases can be treated with these herbs. Every part of the plant, like bark, stems, roots, and

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leaves, can be used for different diseases [4]. *Euphorbia neriifolia* is one of the indigenous medicinal plants of the family "Euphorbiaceae". This plant is most commonly known as the spurge tree. It condemns approximately 7000 species and 275 genera of flowering plants, distributed mostly in the tropical region [10]. Further study on *Euphorbia neriifolia* may increase the standardisation and knowledge of the plant, which could be a panacea to treat some more diseases. *Euphorbia neriifolia* Linn is abundant in the dry, rocky, hilly areas of India's central, north, and south [11]. It is a plant with a full spine. The *Euphorbia neriifolia* plant's leaves are 4-5 mm thick, 15-20 cm long, and contain white latex. Commonly, it is known as milk hedge, thuhar, and sehund. The aerial parts of *Euphorbia neriifolia*, like stem, root, and leaves, are used to treat diseases like ulcers, stomach aches, piles, anaemia, fever, abdominal pain, inflammation, enlargement of the spleen, leucoderma, chronic respiratory trouble, etc. [12]. The white latex of *euphorbia neriifolia* is used for the repair of wounds as evidence of an increase in DNA content, angiogenesis, and tensile strength [13]. The milky latex of *Euphorbia neriifolia* is used as aphrodisiac by the tribal population of Chhattisgarh as an ingredient [14]. The leaf's juice, can be used as a strong purgative for the enlargement of the liver and spleen, leprosy, dropsy, syphilis, general anasarca, etc. when treated with some other ingredients. When this juice is treated with fresh butter as an application for the rectification of unhealthy scabies and ulcers, i.e., it is used for treating the lower abdomen in cases of severe constipation and poisoning. The leaves of *Euphorbia neriifolia* are used as diuretics, aphrodisiacs, and can treat bronchitis, bleeding piles, and ano-rectal fistula [15].

MATERIALS AND METHODS

Collection of plant

The aerial parts, such as leaves and stems, of the *Euphiorbia neriifolia* linn, were collected from suburban areas of Bhopal in the month of March 2021. The aerial parts of plants are used for study and research in phytochemical screening and thin layer chromatography. These collected parts of plants are properly washed in running water and then quenched in distilled water. This cleaning material is then cut into 5-8 cm pieces so it is easy to dry. After that, it is left to dry at room temperature in a shaded and dark place for 8–10 days without being contaminated. The determination of standardisation of *Euphorbia neriifolia* Arial parts was on the basis of suggested parameters by World Health Organization guidelines and the Ayurvedic pharmacopoeia of India. The completely dried materials are then grinded and converted into coarse powder with the help of an electrical grinder. This dried powder of plant material was stored in an airtight container for further studies of TLC and phytochemical analysis.

Defatting of material

Defatting of material is a method of separation of dirt, dust, oil, fat, and other contaminants from plant material so that we get the required material for the process. The defatting is necessary because this contamination can affect the result. For the process of defatting, we leave the coarse powder of plant material of the *Euphorbia neriifolia* plant in petroleum ether for 24 hours at room temperature. After 24 hours, the material is filtered with the help of a spatula, funnel, and filter paper [16], so that the impurities are separated from the required material. Dried the filtered material and stored it in an airtight container.

Chemical reagent

For phytochemical analysis, the useful chemical reagents are Fehling solution A, Fehling solution B, hydrochloric acid, picric acid, ferric chloride, lead acetate, gelatine solution, copper acetate.

Extraction of material by Soxhlet apparatus

The plant material of *Euphorbia neriifolia* Linn was extracted by the use of the Soxhlation method. Here we use solvents like ethanol, petroleum ether, ethyl acetate, chloroform, and water. Phytochemical testing has been done to identify the presence or absence of active chemical constituents or phytoconstituents.

Thin layer chromatography

Thin layer chromatography is a process or technique that is used to separate non-volatile mixtures. This process is performed on a sheet of an inert substrate such as aluminium foil, glass or plastic coated with a very thin layer of adsorbent material. Adsorbent materials include silica gel, cellulose, and alumina. Each solvent extract was treated with thin layer chromatography (TLC) as per the conventional one-dimensional ascending method using silica $60F_{254}$, 7*3cm (Merck) and was cut with ordinary scissors. The marking of the silica plate was done with a pencil. Glass capillaries were used to spot the sample by thin layer chromatography, applying a sample volume of 1 microliter by using capillaries at a distance of 1 cm at 2 tracks. In the solvent system, toluene, ethyl acetate, and formic acid are used in the ratio of 5:4:1. After pre saturation with mobile phase for 20 minutes for development, they are used after the run plates are dried. The movement of the active compound was expressed by its retention factor (R_f); values were calculated for different samples. Thin layer chromatographic studies of the different extracts like chloroform, ethyl acetate, ethanol, and water. The solvent toluene: ethyl acetate: formic acid (5:4:1) was used. Different types of extracts are compared with standard quercitin and then calculated the R_f values. R_f can be clculated by the formula, R_f=Distance travelled by solute / Distance travelled by solvent. Once the chromatogram was developed the R_f value of spot was calculated using the formula and results were shown in (Table 2).

RESULTS AND DISCUSSION

Secondary metabolites such as phenols, alkaloids, flavonoids, terpenoids, quinines, and steroids are present in a large number of plants that are used in pharmaceuticals, pesticides and cosmetics and industries. In the present study, the qualitative test done in all four extracts showed the indication of the presence of metabolites. In the study of the phytochemical investigation, a different phytoconstituents were present in the plant *Euphorbia neriifolia* Linn. extracts. On the basis of research, the alkaloid is absent in all extracts. Carbohydrates are present in water, ethanol and ethyl extracts [17]. Water and ethanol extracts showed the presence of saponins. The phenol test is also absent in all extracts. The flavonoid test is absent in only petroleum ether extract, whereas it is present in chloroform extract, ethyl acetate extract, water extract, and ethanol extracts. The Diterpene test shows its presence in chloroform, water, and ethanol extracts. The Xenthoproteins test is present in ethyl acetate, water, and ethanol extracts. The tannin test is present in water extracts only. On the basis of this study, by identifying these bioactive compounds, some new medicines can be developed that can treat some more diseases or disorders [18]. Presence and absence of different phytochemicals are shown in (Table 1).

Table 1 Test for Phytoconstituents for different extract of *Euphorbia neriifolia*

Secondary metabolites test	Chloroform extract	Ethyl acetate extract	Water extract	Ethanol extract
Alkaloid Test	Absent	Absent	Absent	Absent
Carbohydrates Test	Absent	Present	Present	Present
Saponins Test	Absent	Absent	Present	Present
Phenols Test	Absent	Absent	Absent	Absent
Flavonoids Test	Present	Present	Present	Present
Diterpene Test	Present	Absent	Present	Present
Xenthoproteins Test	Absent	Present	Present	Present
Tannins Test	Absent	Absent	Present	Absent

TLC profiling of all 4 extracts generates some useful results that show the presence of a number of phytochemicals. These phytochemicals give different R_f values in different solvent systems. This disparity in R_f values of the phytochemicals fabricates a very important clue that is useful in understanding of their polarity and also helps in the selection of the appropriate solvent system so that the separation of pure compounds can be done by column chromatography. Compounds showing less R_f value have high polarity, and those with a high Rf value have low polarity. The solvents, when mixed with variable polarity in different ratios, can be used for the separation of pure compounds from extracts [19-20]. Only by analysing the R_f values of compounds in different solvent systems can the selection of an appropriate solvent system for a particular plant extract only be achieved. Thin layer chromatography profiling results are shown in (Fig 1-3).

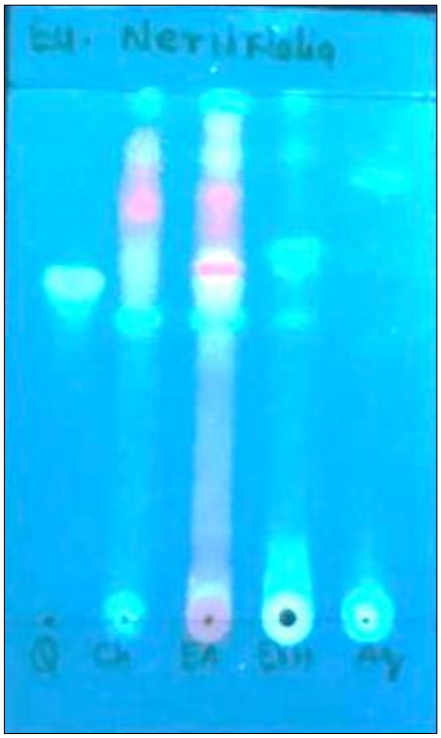

Fig 1

Fig 2

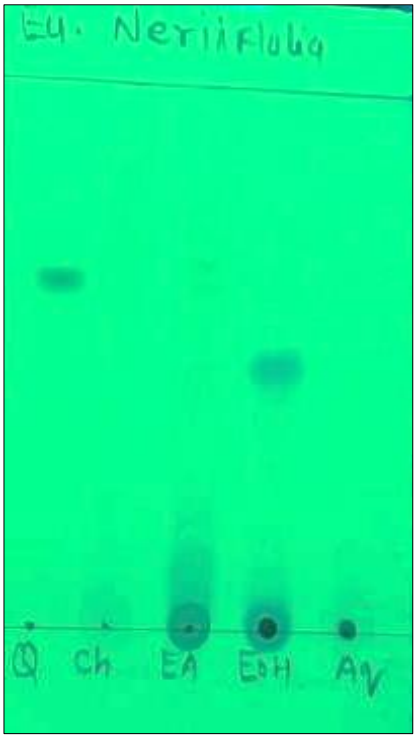

Fig 3

Figure 1 shows long wavelength of ultraviolet light (365nm), the TLC profile in the chloroform extract, ethyl acetate extract, ethanol extract and water extract of *Euphorbia neriifolia*. The solvent system used in TLC is toluene: ethyl acetate: formic acid (5:4:1). R_f values are given in (Table 2).

Figure 2 shows normal visible light of the chloroform extract, ethyl acetate extract, ethanol extract and water extract of *Euphorbia neriifolia*. The solvent system used in TLC is toluene: ethyl acetate: formic acid (5:4:1). R_f values are given in (Table 2).

Figure 3 shows short wavelength of ultraviolet light (254nm), the TLC profile in the chloroform extract, ethyl acetate extract, ethanol extract and water extract of *Euphorbia neriifolia*. The solvent system used in TLC is toluene: ethyl acetate: formic acid (5:4:1). R_f values are given in (Table 2).

In the current circumstances, TLC profiling of all the plant's extracts from stem and leaves in different solvent systems gives an indication of the presence of assorted types of phytochemicals in this plant. Different R_f values of the compound also think about an idea about their polarity. This study will help in the selection of a pertinent solvent system for additional separation of compounds from these plant extracts.

CONCLUSION

Preliminary phytochemical analysis divulged the presence of steroids, flavonoids, and saponins in all the four extracts of the stems and leaves of the *Euphorbia neriifolia* Linn. plant. TLC profiling of plant extracts in different solvent systems authenticates the presence of different groups of phytochemicals.

Table 2 Show the Rf value of TLC solvent system for different extracts of *Euphorbia neriifolia*

Extract name	Total no. of spot detected	Spot detected at long wavelength	R_f value	Spot detected at short wavelength	R_f value	Spot detected at normal light	R_f value
Chloroform	10	6	0.58 0.68 0.78 0.84 0.9 0.98	2	0.66 0.84	2	0.68 0.84
Ethyl Acetate	13	7	0.56 0.62 0.66 0.78 0.86 0.92 0.96	4	0.48 0.62 0.66 0.98	2	0.62 0.66
Ethanol	9	5	0.56 0.64 0.7 0.86 0.84	4	0.48 0.62 0.66 0.72	0	0
Water	2	2	0.54 0.84	0	0	0	0

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