



Extraction, Separation & Identification of Phenolic and Glycoside compounds in leaf extraction of *Murraya Koenigii* (Curry leaves).

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Plants have been one of the most important source of medicines since the beginning of human civilization. Plant-based medications, health goods, pharmaceuticals, food supplements, and cosmetics are in high demand. *Murraya koenigii*, often known as the curry leaf tree, is a multipurpose tree that provides therapeutic goods. This research aims to give a summary of the chemical constituents found in *Murraya koenigii* leaf extract, with a focus on their pharmacological effects. Leaf extracts were used to conduct qualitative and quantitative phytochemicals screening in two distinct solvents: water and methanol. The goal of this research was to find out what phytochemicals components were present in curry leaf extract. The extracts contained glycosides, alkaloids, oils, spooning, cardiac glycosides, phenols, terpenoids, and flavonoids, according to spectral phytochemicals analysis by LC-MS and FTIR. Isolation, purification, and multiplication culture development methods were used to test the extract's antibacterial effectiveness against *Staphylococcus aureus* grime +Ve round shaped bacteria. TLC was performed on *Murraya koenigii* leaf extract, which revealed various Rf values. The extract that yielded promising results was column fractionated, and the obtained fractions were tested for antibacterial and antioxidant activities using a spot assay. The antibacterial and antioxidant activity of *Murraya koenigii* leaf extract demonstrated that plants had both antimicrobial and antioxidant activity, according to the findings.

Key words: Curry leaves , Extraction , Sepration, cottan , Identification , Column Chromatography.

INTRODUCTION

Their medical usefulness, extraction of bioactive chemicals, particularly phenols, has grown in recent years. Phenols, which include phenolic acids, flavonoids, stilbenes are secondary metabolites found in plants. Phenol-rich foods provide superior protection against the development of malignancies, cardiovascular illnesses, diabetes, osteoporosis, and other ailments. These chemicals can be found in varying amounts in fruits, vegetables, spices, cereals, and plants [1–4]. Bioactive components found in herbal plants include lipids, phytochemicals, pharmaceuticals, flavours, perfumes, and pigments [5]. Curry leaves (*Murraya koenigii* L.), which belong to the Rutaceae family, are a popular spice in India. Because of its distinct flavour and scent, it is commonly used in curry preparations [1]. Curry leaves offer antioxidant, antidiabetic, antimicrobial, antibacterial, positive inotropic, and cholesterol-lowering properties [2, 3].

The pharmacodynamic response of *Murraya koenigii* is elicited by a complex interaction of chemical elements. A number of active components with therapeutic characteristics have been isolated and identified. Antioxidant, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic, and cholesterol-lowering properties have been documented for this plant. As a result, the current review highlights the current literature on phytoconstituent isolation, biological activities of isolated compounds, and pharmacological properties of extracts, as well as clinical investigations.

Solid-liquid extraction can be used to extract phenols and glycoside found in plant sources. The extraction procedure is divided into two stages: quick washing and gradual diffusion. Physical and chemical properties of the solute and solvent, as well as solute-solvent interactions are critical to understand when studying the extraction process [4]. Many studies have looked at the solid-liquid batch extraction of phenolic acids from a range of raw materials under varied extraction conditions, including solvent content and particle size. Solid-to-solvent ratio, duration, and temperature [2, 10]. With the help of appropriate models, the kinetics of the extraction process can be accurately predicted [12]. The term "mathematical modelling" refers to the application of mathematics to solve problems.

Curry leaves are rich in calcium, phosphorous, iron, vitamin like C, A, B, E. The leaves have light strong and feebly acidic taste. Even after drying, certain features, like as flavour, taste, and therapeutic properties, will remain. The purpose of this study was to look into the phytochemicals features of curry leaf Extraction.

The goal of this study is to see how solvent concentration and temperature effect total phenol & glycoside recovery from curry leaves using solid-liquid extraction. There is a scarcity of data in the literature on mathematical modeling of total phenols from curry leaves. The purpose of this paper is to look at how solvent concentration and temperature affect the recovery of total phenols from curry leaves using solid-liquid extraction. There is a scarcity of data in the literature on mathematical modeling of total phenols from curry leaves. An attempt was made to examine the extraction kinetics by using the Peleg model and the Power law model to the obtained experimental data in order to get insights into mathematical modeling. In terms of percentae recovery of polyphenols, traditional extraction methods are compared to the novel method.

EXPERIMENTAL

Solvent Extraction: The experiment was done in the aqueous solvent. 100 gm plant source was dissolved in 300 ml solvent 70% aqueous. After the solvent hit on the Bunsen Burner to the solvent is evaporated to half of the solvent and then after the solvent is cool down to the normal temperature. Then the solvent filtered to using Whatman filter paper then kept in an airtight bottle for further experiment.

Identification of phenols: To 2ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution, were added to 1 ml of sample aqueous solution. The presence of phenols was indicated by the formation of a blue or green colour.

Identification of Glycoside: A small amount of alcohol (methanol) was dissolved in sample of aqueous extract of plant leaves and then aqueous Sodium hydroxide was added. Formation of yellow indicated the presence of glycosides.

Isolation by Column Chromatography: Column chromatography was carried out with a stationary phase of silica gel F254 (60-120 mesh) and a mobile phase of solvent. The *L. procumbens* ethyl acetate fraction was eluted with n-hexane, n-hexane: ethyl acetate, and ethyl acetate: methanol (100:90, 70:30, 60:40, 0:100, 95:5, 90:10) in increasing order of polarity to yield 7 fraction. The LC-MS was used to identify the proportion of the isolation column.

Identification test of this Isolation of Phenols: To 2ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution, were added to 1 ml of sample aqueous solution. The presence of phenols was indicated by the formation of a blue or green colour.

Identification test of this Isolation of Glycoside: A small amount of alcohol (methanol) was dissolved in sample of aqueous extract of plant leaves and then aqueous Sodium hydroxide was added. Formation of yellow indicated the presence of glycosides.

Liquid Chromatography-Mass Spectroscopy: The samples were analysed using a Waters ACQUITY UPLC™ system (Waters; Milford, MA, USA), which was linked to a hybrid triple quadrupole-ESI source. A SUNFIRE C18 column (250 mm 4.6 mm, 5μm) and positive mode LC-ESI-MS/MS were used for chromatographic separation. The mobile phase was (A) acetonitrile and (B) 5mM ammonium acetate in 1.5 percent methanol, with a gradient system of 95 percent B in 0–1 minute, 70 percent B in 1–10 minute, 40 percent B in 10–14 minute, 40 percent B in 14–16 minute, 20 percent B in 16–24 minute, 20 percent B in 24–32 minute, 95 percent B in 32–35 minute, 95 percent B in 35–40 minute, 95 percent B in 40–45 minute. Nitrogen gas was employed as the nebulizing and drying gas at flow rates of 30 and 950 L/h. (18) The ESI source potential's capillary voltage was 3.5 kV, and the cone potential was 30 V in each experiment. The source temperature was 125°C, while the desolvation temperature was 350°C. Data was collected and processed using the Mass Lynx V4.1 SCN 714 programme. (19)

Result and Discussion

The presence of peaks that appeared in the chromatogram showed the presence of different compounds from curry leaves water extract. Four of the compounds in the extract indicated by peaks were identified as below

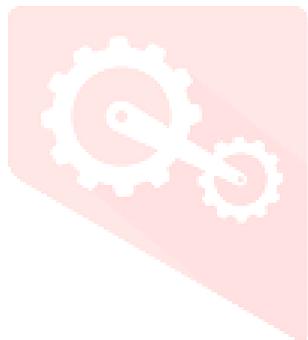
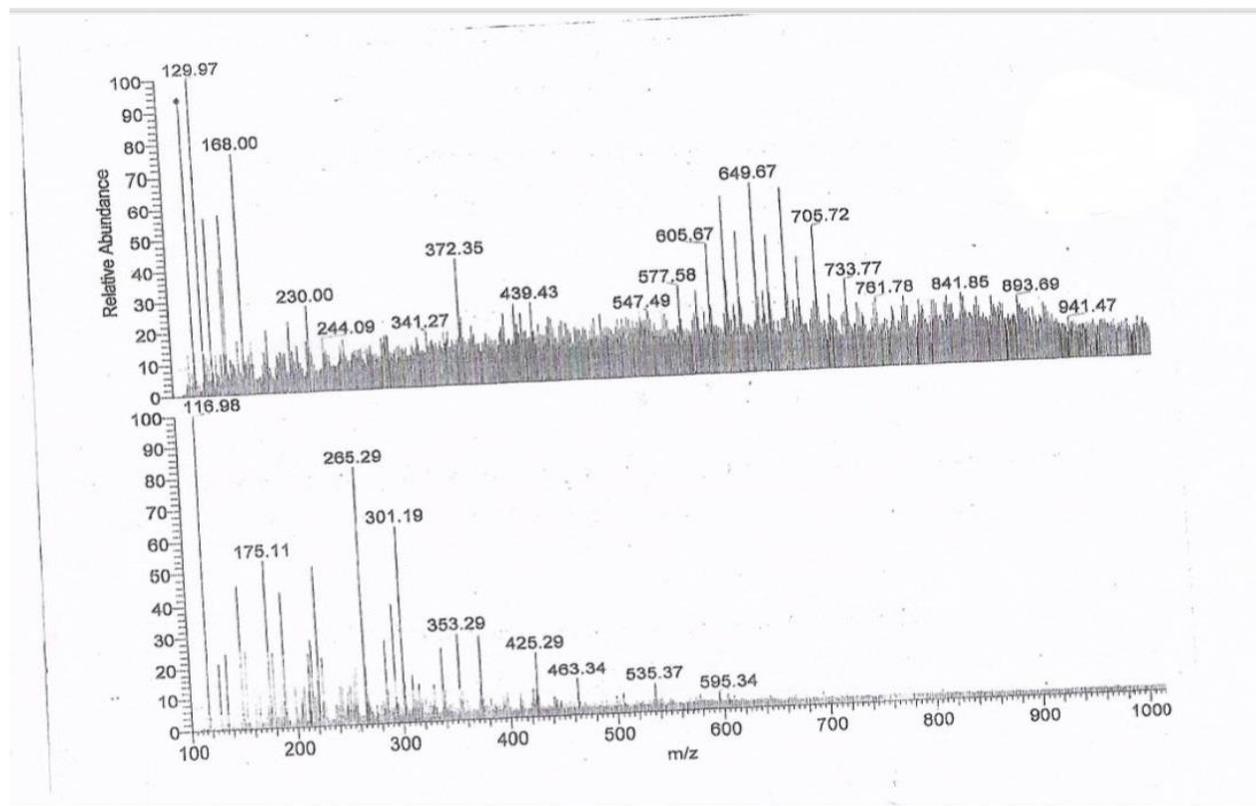
Types of phenol:-

Sr. no	Name of phenols	Molecular formula	M.W. (g/mol)	Relative abundance
1	Phenyl methyl Siloxane oligomer	$(C_2H_6OSi)_n$	547.00	547.49
2	Haloarenes	AR-X	128.30	129.97
3	Benzene sulphonic acid	$C_6H_6O_3S$	158.17	168.00
4	Diazonium Salt	$C_6H_5N_2^+HSO_4^-$	235.22	230.00

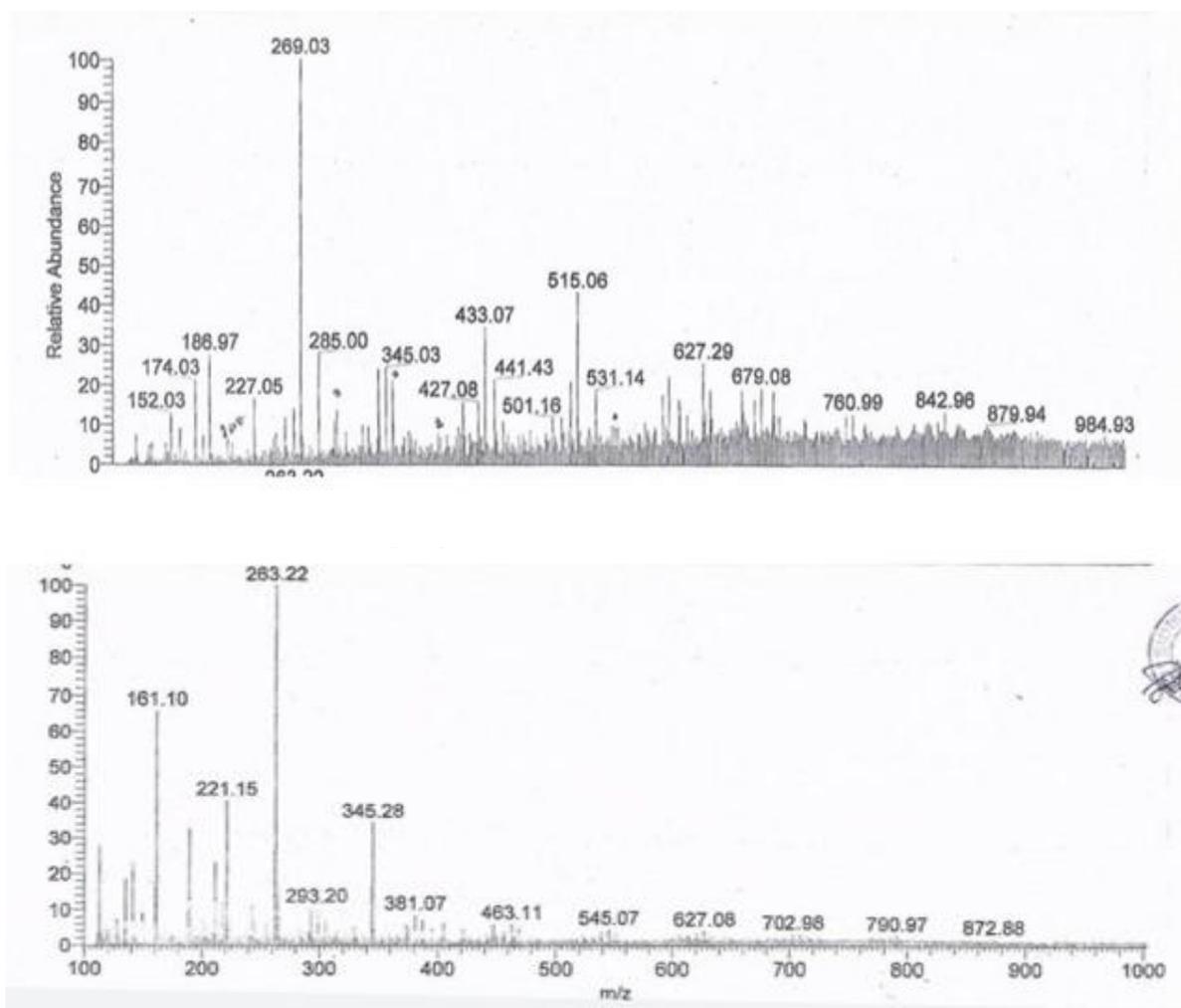
Types of Glycosides:

Sr. No.	Name of Glycosides	Molecular formula	M.W.(g/mol)	Relative Abundance
1	Anthraquinones Glycoside	$C_{59}H_{80}O_{22}$	208.21	208.21
2	Cardiac Glycoside	$C_{86}H_{138}O_{32}$	386.53	386.53
3	Coumarin Glycoside	$C_{54}H_{80}O_{20}$	539.13	539.13
4	Cyanogenic Glycoside	$C_{57}H_{83}O_{24}N$	353.32	353.32
5	Flavonoid Glycoside	$C_{60}H_{84}O_{20}$	318	318

Phenols LC-MS graph:



Glycoside LC-MS Graph:

**Anti Microbial activity:**

Curry leaves extraction were tested for their antimicrobial activity against *E. coli* (10^6) using core borer plates methods (pati et al 2012)²¹ at 100 ppm (10mg/ml) concentration in aqueous solvent. After the identification biological application checked in terms of antimicrobial activity which shows good results.

Conclusion:

The water extract prepared from the *Murraya Koenigi* leaves used for the identification of phytochemicals. By performing various chemical tests it shows presence of flavonoids, terpenoids, glucosides, phenols, etc... from the present phytochemicals phenols were isolated by column chromatography. The isolated phenolic fraction is further analysed by LC-MS. Chromatography from the LC-MS. We can confirm the presence of phenols, Phenyl methyl Siloxane oligomer, Haloarenes, Benzene sulphonic acid, Diazonium Salt. After the identification biological application checked in terms of antimicrobial & antifungal activity which shows good results.

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