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PHYTOCHEMICAL ANALYSIS AND ISOLATION OF FLAVONOID COMPOUND FROM METHANOL EXTRACT OF THE LEAVES OF *SCURRULA PARASITICA* L.

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Keywords:

Scurrula parasitica L., Column chromatography, Thin layer chromatography, HPLC, Quercetin, mass spectral analysis

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ABSTRACT: *Scurrula parasitica* L. is a parasitic plant, traditionally used by the people of Mizoram for the treatment of various diseases, and locally called Vatesawi. The bioassay-guided purification of the methanol extract of the leaves of *Scurrula parasitica* L. led to the isolation of 2-(3, 4-dihydroxy phenyl)-3,5,7-trihydroxy-4H-Chromen-4-one. Quercetin is a flavonol that belongs to one of the subclasses of flavonoids. For separation and purification of compounds, thin layer chromatography and column chromatography were performed using the plant extract. Then identification was done by high-performance liquid chromatography (HPLC) with mass spectral analysis, and confirmation of the isolated compound was performed with thin layer chromatography. The results showed that the solvent system of ethyl acetate 7: chloroform 3 had accurate separation, and the fraction no-200 from column chromatography was processed for identification of the compound. Structures were established with the mass spectrum and confirmation of the compound was done by comparing with standard quercetin, and both appeared at the same wavelength of 254nm (RT 2.63).

INTRODUCTION: Medicinal plants possess therapeutic properties against certain diseases or serve as the origin of useful drugs [1-2]. The true efficacies of the substances in medicinal plants have not been recognized until the 19th century. Plants possess active compounds that have proven to be useful in numerous medical fields for centuries [3]. These phytochemicals are the major principles in curing human ailments. Different plants contain certain active principles in them; these active ingredients may differ even in different organs like root, bark, leaves, seeds, twigs and fruits of the same plant.

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The substances derived from plants are used for the manufacture of synthetic drugs [4]. Medicinal plants are rich in secondary metabolites; thus modern pharmaceutical industries have tried to isolate these potential bioactive compounds for the treatment of various diseases.

Scurrula parasitica L. is a parasitic shrub found growing on *Dendrophthoe falcate, Myrica esculenta,* and *Mangifera indica.* It belongs to the family Loranthaceae. It is found in Sri Lanka, Nepal, Bhutan, Bangladesh, Myanmar, Thailand, Taiwan, Vietnam, Indonesia, Philippines, Moluccas, and Timor. In India, it is distributed all over the Western Ghats regions of Maharashtra and parts of Uttar Karnataka and Tamil Nadu. The plant is used as a traditional medicinal herb as an antioxidant and against microbial infections, hypertension, and cancer in Asia [5]. In Java and Indonesia, the infusion of *Scurrula parasitica* L. is used for the treatment of cancer [6].

In southern China, the leaves and stem of *Scurrula parasitica* L. are used as antineoplastic, cardio-tonic, as an antioxidant and for treatment of Schizophrenia [7-8]. The plant shows anti-diabetic, cytotoxic, anticancer, anti-hepatoxic and immuno-modulatory activity [9]. Recently it was observed that the leaves of *Scurrula parastica* L. were frequently used by the tribal inhabitants of Mizoram for the treatment of diabetes. 21 days anti-hyperglycemic experiment using streptozotocin-induced diabetic rats was performed with two doses of the plant extract, and the results show a significant lowering of blood glucose, cholesterol, LDL, triglyceride, SGOT, ALP, and SGPT, along with an increase in HDL and body weights in rats. 5 anti-diabetic compounds were also detected, which might be the reason for its anti-hyperglycemic properties [10]. Important phytochemicals responsible for numerous biological activities like terpenoids, flavonoids, alkaloids, viscotoxins, amines, lectins have been tested to be present on *Scurrula parastica* L. [11-12] This paper focus on the ethnomedicinal claims and isolation of active compound, extracted from the leaves of *Scurrula parasitica* L. Isolation and identification of active compound is done through standard techniques like chromatography and spectral methods [13].

MATERIALS AND METHODS:

Plant Material and Extraction: The leaves of *Scurrula parasitica* L. were collected from Kolasib, Mizoram. Taxonomic identification was done at the Botanical Survey of India, Shillong (No: BSI/ ERC/ Tech/ 2017/ 43), and authentication was done at the department of environmental science, Mizoram University, with a voucher number of MZU 742. Fresh leaves of *Scurrula parasitica* L. were air-dried at room temperature and prepared into a coarse powder, and stored in a beaker. Approximately 200gm of the plant powder was weighed and subjected to continuous hot extraction using the Soxhlet apparatus at 60°C. The extraction was carried out successively using petroleum ether, chloroform, and methanol. Subsequently, the extracts were evaporated under pressure using a rotary evaporator until all the solvents have evaporated to give pure crude extracts. The methanol extract was used for the experiment.

Phytochemical Screening of the Crude Extract: Preliminary phytochemical screening was performed to detect the presence of important secondary metabolites in the crude extract of *Scurrula parasitica* L. The alterations in color and formation of precipitates in the plant extracts performed in separate test tubes denote positive results. Observations were recorded.

Thin Layer Chromatography: Thin-layer chromatography (TLC) is a method of separation in which differential migration of solvents occurs along with a fine powder spread on a glass plate. Several compounds are spotted on a thin layer of solid adsorbent, then the compounds along with the mobile phase travel to distances varying on the particle coefficient of each molecule. Readymade aluminum-coated silica gel plates (Merck and Co. Ltd.) were used for the research. The plate is cut into smaller pieces into a 4 × 10 cm dimension. The plates are kept in an oven for 120°C for half an hour before use [14]. With the help of capillary tubes, 2.5µl of plant extracts are spotted carefully on the silica gel plate. Spotting was done 1 cm above the bottom of the plate and kept in a development chamber. The lid of the development chamber was closed to avoid tailing, and the solvent front was then allowed to travel upwards up to a distance of $3/4^{th}$ from the baseline, and then the lid of the chamber is opened, and the plate is removed and allowed to dry in the air. The plate is then kept in an iodine chamber for a few minutes. The solvent system is based on elusive power because the rate of migration of compounds on a given adsorbent depends on the solvents being used [15-16].

Column Chromatography: The column chromatographic technique is most commonly used for the separation of compounds into several fractions according to the affinity or solvating capacity of the compounds to the solvent used. The study involves fractionation and isolation of compounds from pharmacologically active ethanol extract. The structure of the compound was tried to establish by spectroscopic methods. A Glass column of a 20 mm dimension was taken and held firmly with the help of a stand and fitted with a stopcock. Solvent system of Ethyl acetate: chloroform (7:3, 8; 2 and 9:1) was used. The stationary phase was made with Silica gel (60-120 mesh size), which was heated at a maximum temperature of 120 °C and placed in the column. Cotton is inserted into the column and the bottom

portion is plugged and the silica gel (60-120 mesh size) is packed into the column which is done by the wet package method. Then the stationary phase is allowed to settle down without entrapment of air bubbles [17]. The column is allowed to stand uniformly without disturbances for two hours. Fractionation of extract was carried out using the above solvent system to obtain the bioactive compound. Fractions were collected, and TLC was performed and similar fractions were pool together.

Preparative Thin Layer Chromatography: IPPC or Preparative planar (thin-layer) chromatography is used for the isolation/ separation of compounds. The main purpose is the production of 10 to 1000mg of the separated compounds for structural identification like mass spectrum, Infrared/ Ultraviolet, and for analysis of other activities. Glass plates were used, in which the glasses were cut into 6x4 inch dimensions. A total of 10 slides were made for the experiment.

The glasses were washed properly with water and dried in the oven. Then, Silica gel 'G' as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes; this suspension was layered over a glass plate which was air-dried until the transparency of the layer disappeared. The plates were dried in a hot air oven at 110 °C for 30 min and then stored in a dry atmosphere and used whenever required.

Mass Spectral Analysis: The mass spectrum of the isolated bioactive compound was analyzed using LCMS Accucore (C18, 150 × 2.1, 2.6um), SAIF, CSIR-CDRI, Lucknow, India. Then the molecular weight of the peak compound given by the spectrum was integrated with the NIST database online library for confirmation of the specific compound.

High-Performance Liquid Chromatography: HPLC was performed with Quaternary LC Pump Model 200Q/410 with series 200 Autosampler. The mobile phase was determined based on an isocratic system was using appropriate solvents to detect the wavelength, and then it was incorporated with a standard compound. Both the standard and isolated compounds were eluted together. The peak and retention time were observed. The final result obtained was then overlapped.

Thin Layer Chromatography Analysis for Isolated Compound: Further, the isolated compound was subjected to TLC analysis for compound confirmation. Both the standard and isolated compounds were eluted, and movement was observed up to a certain point. It was then visualized under Iodine dye in a closed chamber.

RESULTS:

Phytochemical Screening of the Crude Extract: The preliminary phytochemical screening of the leaves of *Scurrula parasitica* L. revealed the presence of important bioactive components alkaloid, flavonoid, phenols, saponin, tannin, terpenoids, reducing sugar and phytosterols in the methanolic extract and absence of glycosides.

Thin Layer Chromatography: The results of thin-layer chromatography of methanol extract of *Scurrula parasitica* L. are presented in **Table 3**. A definite spot showing yellowish-red was detected in the solvent system of ethyl acetate and chloroform (7: 3) and Ethyl acetate: Chloroform: Methanol (7:2:1) after keeping it inside an iodine chamber. But ethyl acetate and chloroform (7:3) display the best result. Thus, it was selected to be used as a solvent system.

TABLE 1: THE SOLVENT SYSTEM USED IN THIN-LAYER CHROMATOGRAPHY

S. no.	Solvent system	Results
1	Methanol	No definite spot
2	Methanol: Chloroform (6:4)	Spots detected, tailing
3	Ethyl acetate	A single spot, tailing exists
4	Chloroform	No definite spot
5	Ethyl acetate: Chloroform (7:3)	Single spot with R_f value of 0.44 mm
6	Ethyl acetate: Chloroform: Methanol (7:2:1)	Definite spots with an R_f value of 0.50, but tailing exists

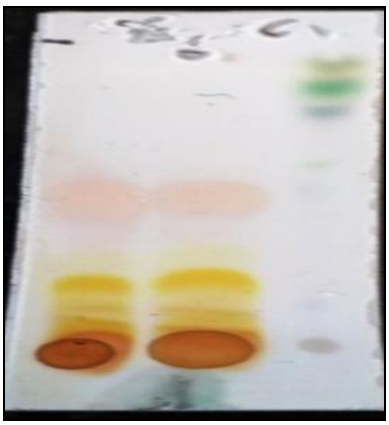

FIG. 1: ETHYL ACETATE: CHLOROFORM (7:3)

Column Chromatography: Around 200 to 250 fractions were collected and recorded, in which fractions of the elutes namely F 1 – 37, F 38 – 76, F 77 – 108, F 109 – 146, F 147 – 168, F 169 – 204 all shows single spots and are combined and transferred to a small beaker evaporated to dryness. The pooled fractions were named A, B, C, D, E, and F, respectively. The elutes were then kept in a refrigerator for 12 hours to enhance purification.

According to fractions collected from column chromatography, Fraction No. F-200 with TLC profile of ethyl acetate: chloroform (7:3) obtaining a single spot with an R_f value of 0.44 mm showed the best results and was chosen for further analysis.

Preparative Thin Layer Chromatography: From the results of column chromatography, the solvent system of ethyl acetate 7: chloroform 3 was prepared and quantification was done on the TLC plates. The process was continued until a substantial amount of the compounds were available for further analysis. Then the compound was selected for Mass spectral analysis for identification. Both HPLC and TLC analyses were done for isolated compound confirmation.

Identification of the Isolated Bioactive Compound: Identification of the isolated compound was done by Mass spectral analysis, HPLC, and TLC along with the NIST database.

Mass Spectral Analysis: The ESI-Mass spectrum of the sample (full positive scan mode) indicates the molecular peak at *m/z* 302.23 $[M+H]^+$. Further, the molecular weight of the isolated compound was incorporated with the NIST database and found to be 302.23, which is Quercetin. The compound was further confirmed by matching with NIST Database online library.

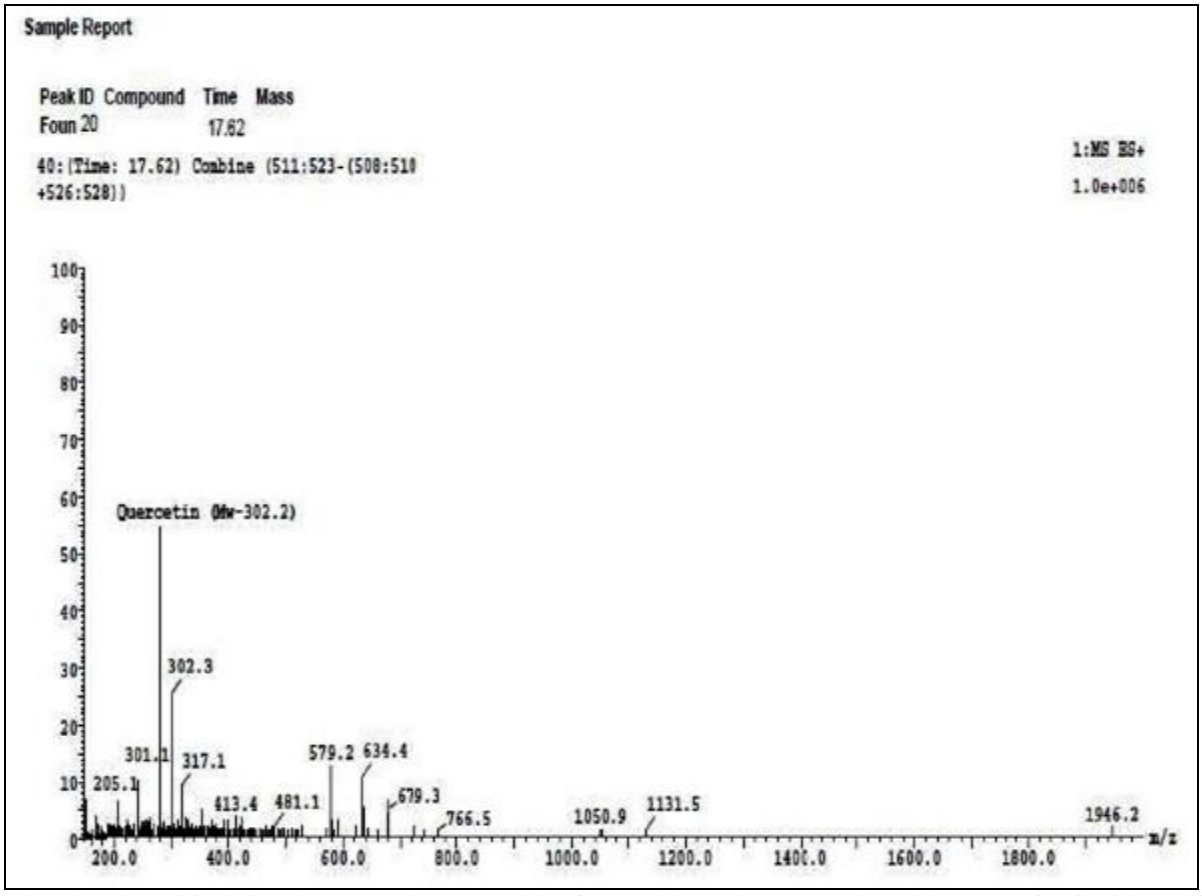

FIG. 2: LC-ESI-MS/MS SPECTRUM OF THE ISOLATED COMPOUND FROM *SCURRULA PARASITICA* L.

HPLC for Confirmation of Isolated Bioactive Compound: HPLC was accomplished with an Isocratic system (A) 60% Acetonitrile and (B) 40% Methanol (60: 40). The detection wavelength was 254nm. The isolated compound was incorporated with standard Quercetin obtained from HiMedia (RM6191-25g). The compound was confirmed by HPLC with standard Quercetin at a wavelength of 254nm (RT 2.63). This was followed by TLC using a similar solvent system.

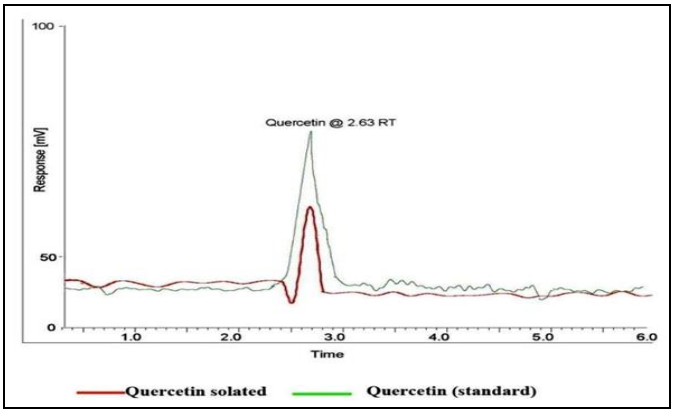

FIG. 3: HPLC ANALYSIS OF ISOLATED COMPOUND FROM *SCURRULA PARASITICA L.* AND QUERCETIN

Thin Layer Chromatography Analysis for Isolated Compound: Finally, Quercetin and the isolated compound were eluted by the TLC method, and the movement was observed up to a certain point. Both appeared at the same level.

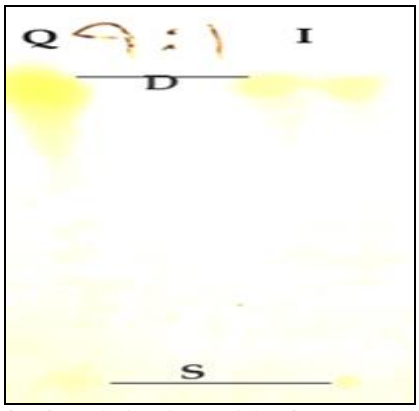

FIG. 4: ANALYSIS OF ISOLATED COMPOUND FROM *SCURRULA PARASITICA* L. S- Spotted area, D- detection level, Q- quercetin, I- isolated compound.

DISCUSSION:

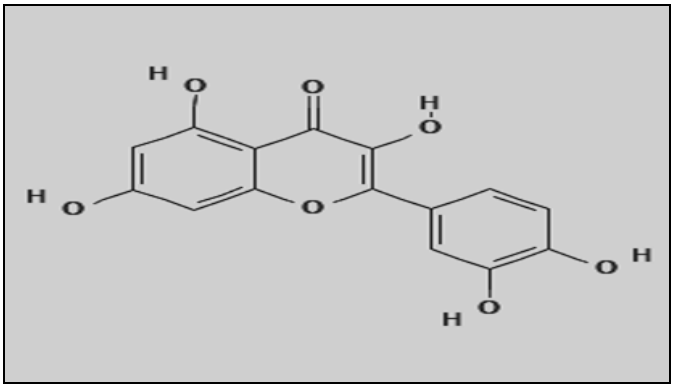

FIG. 5: CHEMICAL STRUCTURE OF QUERCETIN

Quercetin is characterized as a flavonol that belongs to one of the subclasses of flavonoid compounds. IUPAC Name is 2-(3, 4-dihydroxy phenyl)-3,5,7-trihydroxy-4H-Chromen-4-one.

The molecular formula of quercetin is $C_{15}H_{10}O_7$ and has a molecular weight of 302.23 g/mole. Quercetin is aglycone lacking an attached sugar. It is yellowish, hardly soluble in hot water, insoluble in cold water and quite soluble in alcohol solvents. This bioflavonoid has shown significant results in lowering blood pressure, endothelial functions and inflammations [18-20]. Quercetin also displays beneficial effects in protein oxidation-reduction and LDL-C [21-22]. Numerous investigations of quercetin in guinea pigs through oral administration or inhalation have shown anti-asthmatic activity [23-25]. Intake of non-tea quercetin reduces the risk of developing colon, however not rectal cancer [26]. Supplementation of quercetin reduces the development of cardiac hypertrophy in rats [27]. Quercetin induces a progressive and sustained reduction in blood pressure in rat models of hypertension and metabolic syndrome [28]. *In-vitro* antibacterial activity of quercetin proved to be effective against microorganisms associated with the start and progression of periodontal diseases like *Actinomyces naeslundii* wvl, *Actinomyces viscosus*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* [29-30]. Quercetin demonstrates *in-vitro* antiviral activity against parainfluenza virus type 3, poliovirus type 1, HIV transcriptase, hepatitis C and respiratory syncytial virus[31-32].

CONCLUSION: Thin layer chromatography of the methanol extract of the leaves of *Scurrula parasitica* L. results indicates that the solvent system of Ethyl acetate 7: Chloroform 3 proved to be the most accurate. The fraction (Fraction no 200) from column chromatography was further processed for identification. Then, confirmation was done in which the isolated compound was compared to standard quercetin HiMedia (RM6191-25g), and they both appeared at the same wavelength of 254nm (RT 2.63). The isolation of quercetin from the methanol extract of the leaves of *Scurrula parasitica* L. will prove to be useful in the manufacture of biopharmaceutical products in the future.

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