Qualitative Phytochemical screening and elemental analysis of Thai basil leaves using SEM-EDS, XRD

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ABSTRACT

Thai basil leaf (*Ocimum bailicum var. thyrsiflorum)* contains various compounds such as flavonoid, alkaloid, phenol and essential oil, so it needs to be fractionated to find out the chemical substances that have a definite physiological action on the living system. The qualitative analysis is very essential to identify the phytochemical constituents present in medicinal plants. In present study deals with the preliminary phytochemical screening of petroleum ether extract of Thai basil leaves followed by qualitative analysis. Morphological structure and elemental composition was done by using SEM-EDS. X-ray diffraction (XRD) technique used to determine the crystallographic structure of a Thai basil leaves. Phytochemical analysis revealed the presence of several bioactive compounds such as flavonoids, phenols, alkaloids, steroids, tannins and saponin. Scanning electron microscopy (SEM) image indicate the morphology of Thai basil leaves. EDS characterization indicates that they have good purity with fewer amounts of impurities. Energy dispersive spectroscopy (EDS) data indicates the Carbon content was 39.70 % and Oxygen content was 49.03 %. XRD indicates the amorphous structure of Thai basil leaves.

Keywords: Phytochemical screening, SEM, EDS, XRD, Thai basil leaves.

INTRODUCTION

Herbs provided us with some of the very important lifesaving drugs used in the armamentarium of modern medicine [1]. Some world's population depends on traditional medicine because of scarcity, high cost of orthodox medicine and unpleasant side effects. Phytochemical screening is to isolate various constituents of the plants for assessing their biological activity or medicinal uses [2]. The medicinal value is the plants are due to the presence of particular chemical substances that have a definite physiological action on the living system. Thai basil plant genius *Ocimum*, family Lamiaceae, which are rich in phytochemicals like alkaloids, flavonoids, tannin, steroids, saponins, and phenols are very useful for their therapeutic potentials [3]. Several studies have shown various activities of *Ocimum* species including bactericidal, anti-inflammatory, antioxidative, and anticancer, for cough and kidney malfunction, hypoglycemic, nervous system stimulation and protection from radiation [10][11]. More than 75% of the world population depends upon medicinal plants for their basic health needs. Plant-based medicine has become a popular alternative for synthetic medicine because it does not cause any adverse effect [12]. The pharmaceutical potentiality of *Ocimum* species may be attributed to their profound biological effects due to the presence of active polypehnols, as hydroxycinnamic acids (caffeic acid and rosmarinic acid) and flavonoids, mainly in the form of derivatives such as esters and glycosides [4]. An interesting plant which belongs to genus *Ocimum* is *Ocimum basilicum var. thyrsiflorum* which is native to Southeast Asia and is cultivated in other regions of India, Asia, Africa, the Mediterranean region and all over the world [3]. Thai basil is sturdy and compact, growing up to 45 cm (1 ft. 6 inches), and has small, narrow leaves, purple stems, and pink-purple flowers. Thai basil is a tender perennial but is typically grown as an annual.

The aim of present study was to investigate the bioactive constituents of Thai basil leaf extract. Morphological structure with elemental composition of Thai basil leaves (*Ocimum basilicum var. thyrsiflorum*) growing in Lucknow, India was carried out.

2. MATERIALS AND METHODS

2.1 Sample collection

The sample of Thai basil leave was collected by hand picking from Babasaheb Bhimrao Ambedkar University Lucknow, U.P, India on 18 January, 2022.

Figure 1: Thai basil plant

2.2 Sample preparation

The collected sample was cleaned well with tap water to remove all the dust. The cleaned sample was once again washed with distilled water to avoid any cross contamination of the sample and the sample was air dried under the sun for 3 days at 27- 30^0C and then subjected to electrical grinder to obtain coarse powder and then stored in air tight container to avoid it form an attack or certain environment . Thereafter the dry powder used for further extraction and various processes.

2.3 Extraction using Soxhlet Unit

The extraction of plant material was performed at the lab of Food science and technology, Babasaheb Bhimrao Ambedkar University Lucknow, U.P, India. Extraction of Thai basil leaves has done by using soxhleation method. In this method, the processed sample powder was subjected to the extraction in the soxhlet unit using the petroleum ether as solvent [5].

Briefly 10g of powder sample was taken for the process of extraction with 250ml of petroleum ether as solvent. The boiling temperature of the soxhlet unit was maintained to 60-70^0C. The flask containing the extraction solvent was heated to reflux. The process of extraction was carried out for 3 days (15-20 cycles). After the process of extraction, the sample was collected from the distillation flask and subjected to the process of filtration using whatman filter paper and followed by the evaporation using the water bath to allow the solvent to get evaporated and to obtain the concentrated sample with can be used for further experimental processed.

2.4 Phytochemical screening

Phytochemicals are the chemical compounds which are produced by the plants. Preliminary qualitative screening for phytochemicals, of Thai basil leave extract was carried out with following method [6].

Test for Coumarins

2 ml of extract was treated with 3 ml of 10% NaOH. Observed the formation of yellow colour indicating the presence of coumarins.

Test for Steroids (Libermann Burchard Test)

1 ml of extract was dissolved in 10 ml of chloroform. To this mixture equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer becomes red while lower layer of sulphuric acid turns yellow in colour with green fluorescence indicating the presence of steroid.

Test for Saponins (Foam test)

2 ml of extract was taken in a test tube and 6 ml of distilled water was added to it. The mixture was then shaken vigorously. The persistence of foam was observed that indicates the presence of saponins.

Test for Terpenoids (Salkowski test)

2 ml of extract was treated with 2 ml of acetic anhydride. Few drops of concentrated sulphuric acid was then added to this solution and observed the formation of blue, green rings that indicates the presence of terpenoids.

Test for Quinones

1 ml of extract was added to the 2 ml of dilute NaOH. Formation of blue green or red coloration confirms the presence of quinones.

Test for Tannins (Braymer's test) 2 ml of extract was allowed to react with 10% alcoholic ferric chloride solution. Formation of blue or greenish colour of the solution was observed. This was the indication of the presence of the tannins.

Test for Flavonoids (Alkaline reagent test)

2 ml of extract was treated with few drops of 1N sodium hydroxide solution and observed the formation of intense yellow colour. This yellow colour becomes colourless on addition of dilute hydrochloric acid, indicating the presence of flavonoids.

Test for Alkaloids (Mayer's Test)

2 ml of extract was treated with 2 drops of Mayer's reagent. Presence of white creamy precipitate indicates the positive test.

2.5 SEM-EDS and XRD analysis-

2.5.1 Scanning Electron Microscopy (SEM)-Energy Dispersive X-ray Spectroscopy (EDS) Analysis-

The Scanning Electron Microscopy (SEM) JEOL, model- JSM 6490 LV ,Japan available at University Science Instrumentation Centre (USIC), Babasaheb Bhimrao Ambedkar University Lucknow, U.P, India. Energy dispersive X- ray spectroscopy INCA- act model-

51-ADD0013, Oxford, instrument where used for understanding morphological, structure feature and elemental composition of Thai basil leaves [8]. Briefly for SEM analysis, sample were mounted on aluminium stub using double sided carbon tape, then sample were coated using sputter coater model- (JEOL JFC- 1600) auto fin coater with an excel rating voltage of 10KV at high vacuum (HV) mode and secondary electron image (SEI). Typically setting at a magnification at $\times 1000$ (10 μm) for a sample at study. The semi quantification elemental analysis to identify the weight % of major and minor elements present in the sample where done using OXFORD INCA energy dispersive X – ray spectrometer (EDS).

2.5.2 X-Ray Diffraction Analysis-

Powder X-Ray Diffraction pattern were recorded on an X-Ray Diffractometer (XRD), [7] Bruker, model-D8 advance Eco, Germany available at University Science Instrumentation Centre (USIC), Babasaheb Bhimrao Ambedkar University Lucknow, U.P, India. XRD analysis is a technique used to determine the crystallographic structure of a material [9]. Briefly, the data was collected in the 2 Ɵ ranges. Sample were prepared for x-ray diffraction by grinding to powder form\ tale (<0.062mm) then place amount of sample in the centre of sample holder for XRD Analysis. After that loading the sample and then scanning.

3 RESULTS AND DISSCUSSION

3.1 Phytochemical Analysis of Thai basil leaves extract

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body [6]. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example Saponins protect against hypercholesterolemia and antibiotic properties. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [9]. Alkaloids protected against chronic disease. Steroids show the analgesic properties. The steroids and saponins were responsible for central nervous system activities.

The present study carried out the Thai basil leaf extract revealed the presence of medicinal active constituents. The phytochemical active compounds of Thai basil were qualitatively

analysed for leaf separately and the results are presented in Table 1. In phytochemical screening process revealed the presence (+) of alkaloids, flavonoids, steroids, phenolic compounds, saponins, tannin and the negative (-) result of coumarins, terpenoids, quinone, cardiac glycosides.

TEST	INFERENCE	PETROLEUM ETHER EXTRACT
Test for tannin	**Formation of blue greenish colour**	+
Test for saponins	**Formation of persistence foam**	**+**
Test for quinone	**Yellow precipitate**	**-**
Test for flavonoids	**Formation of white precipitate**	**+**
Test for alkaloids	**Presence of green colour or white precipitate**	**+**
Test for glycoside	**Brown ring at the interface**	**-**
Test for terpenoid	**Formation of intense colour**	**-**
Test for coumarin	**Formation of yellow colour**	**-**
Test for phenol	**Formation of dark blue/intense colour**	**+**
Test for steroid	**Formation of green colour**	**+**

Table 1: Qualitative phytochemical screening of essential oil extracted by Thai basil leaves. (+) indicates 'presence', (-) indicates 'absence'.

Figure 2: Results of phytochemical screening

3.2 SEM-EDX and XRD analysis-

3.2.1 SEM (Scanning electron microscope)-

Morphology of Thai basil leaves has been studied by SEM technique at different magnification shown in Figure 3,4,5,6 Thai basil leaves powder presents Agglomerated structure and highly branched like structure at magnification X500, X1000, X2,500.

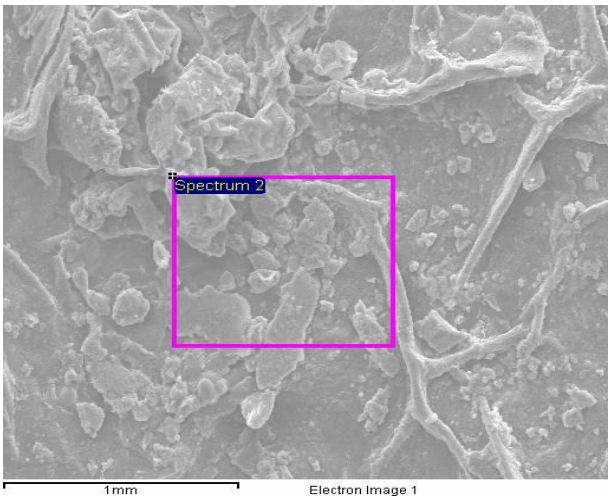

Figure 3: Showing 1mm electron image

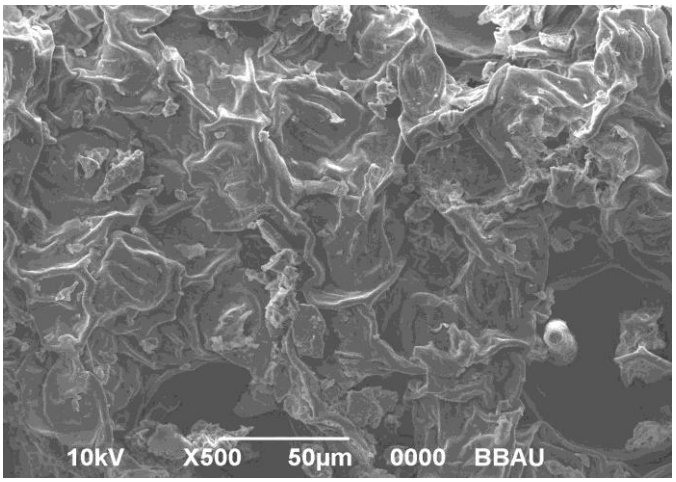

Figure 4: Showing 50-500X µm image

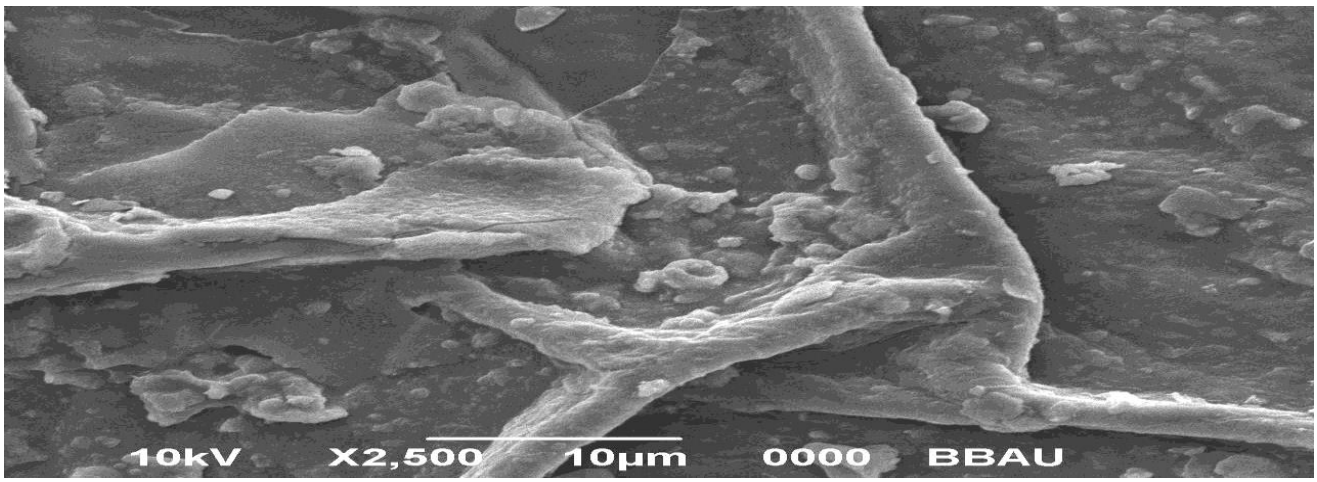

Figure 5: Showing electron image at 10µ-X2,500

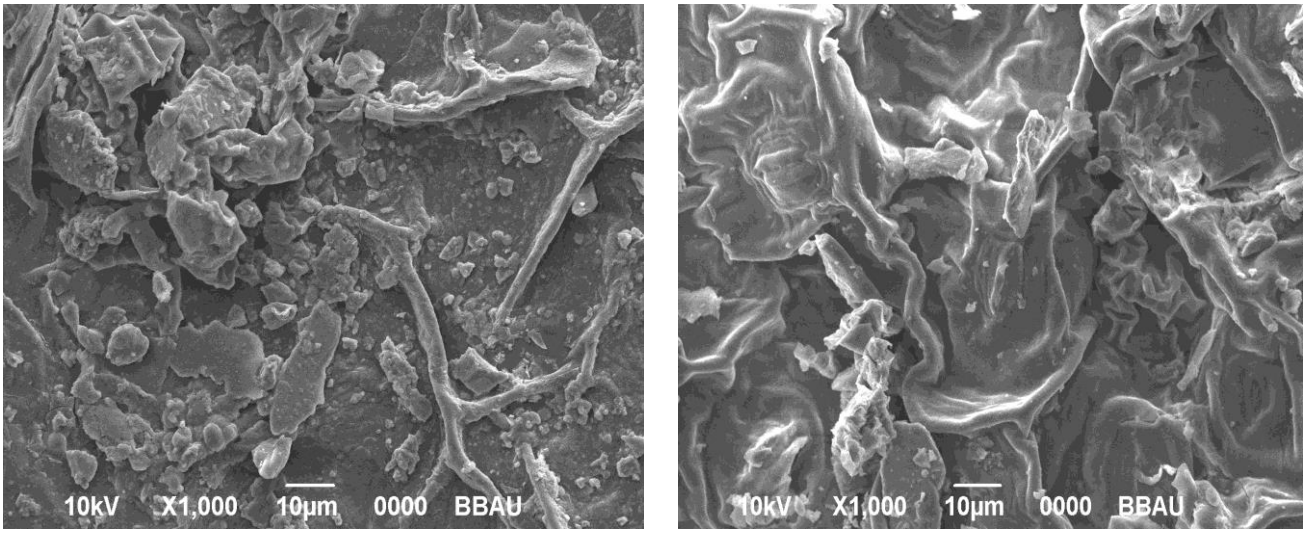

Figure 6: Showing the two electron image at 10 µm – X1,000

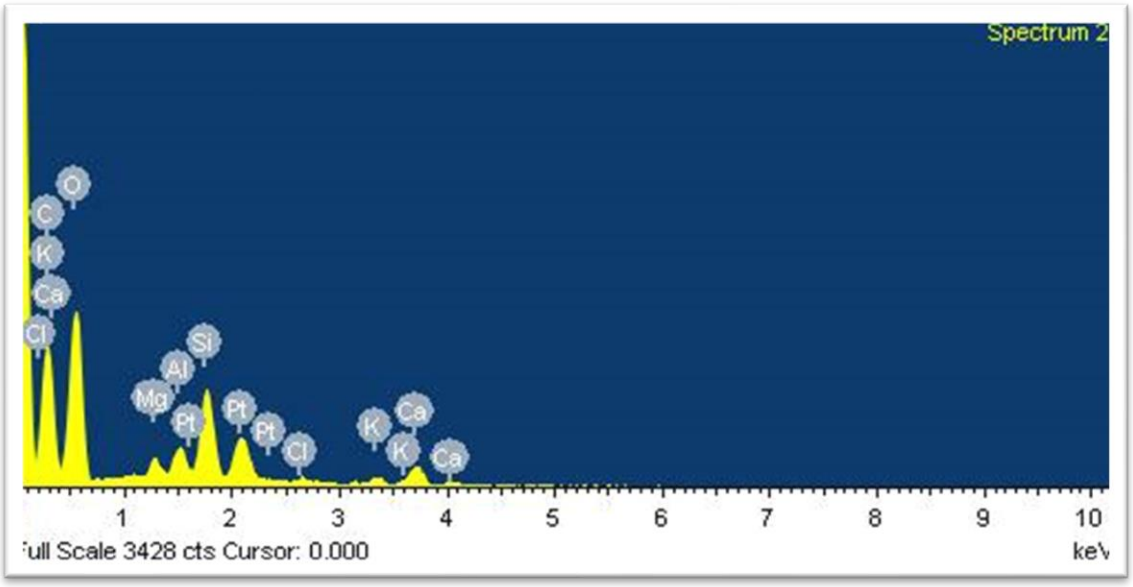

Figure 7: Showing EDX graph of element composition of Thai basil leaves powder

Element	Weight%	Atomic%
C K	37.70	48.20
O K	49.03	47.06
Mg K	0.97	0.62
Al K	1.35	0.77
Si K	4.00	2.19
Cl K	0.22	0.09
K K	0.47	0.18
Ca K	1.33	0.51
Pt M	4.93	0.39
Totals	100.00	

Table 2: Showing the elements composition present in Thai basil leaves powder

EDS studies of Thai basil leaves resulted in detection of 9 elements shows in Figure 7 including Carbon, Oxygen, Magnesium, Aluminium, Silicon, Chlorine, Potassium, Calcium and Platinum in the Thai basil leaves. The EDS characterization indicates that the Thai basil leaves has good purity i.e. Oxygen content 49.03%, Carbon content was 37.70% with very less impurities. There were few traces of impurities present in the EDS spectra.

4.3.2 XRD analysis –

Analysis the nature of Thai basil dry leaves powder by using XRD (X-ray diffraction). The XRD pattern was amorphous with short range ordering. Material was very broad humped peak showing. The amorphous structure of Thai basil leaves has been showed in Figure 8 at (WL= 1.54060) in the Bragg reflection 2θ range from 10^0 to 80^0 respectively.

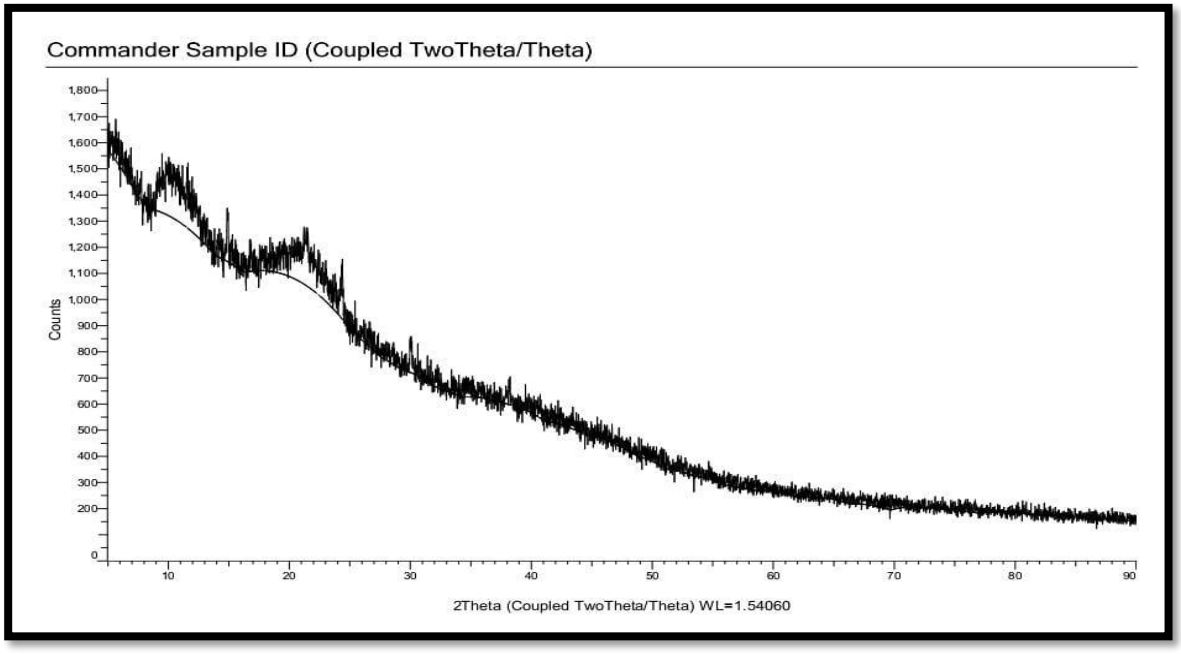

Figure 8: Graph showing the XRD pattern synthesize Thai basil dry leaves powder

CONCLUSION

The study revealed presence of several phytochemicals present in the extract (flavonoid, alkaloids, Tannin, saponin, glycosides, phenols and steroids. Phytochemical analysis of Thai basil leaf extract resulted in identification of various phytochemicals having medicinal and pharmaceutical importance. SEM-EDS analysis showed that leaves of Thai basil are good source of elements like carbon, oxygen, magnesium, potassium, aluminium, silicon and

calcium. The elements content makes the plant to be rich in curing many diseases and ailments. The percentage of silicon weightage was 4.0 % and carbon and oxygen weightage was very high as compare to other element. In X-Ray Diffraction shows the crystallographic structure of a material. X-ray diffraction analysis result shows the Thai basil leaves are amorphous structure.

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CONFLICT OF INTEREST

The authors declare that they have no known conflict of interests or personal relationships that may affect the work reported in this paper.

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