Section A -Research paper
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ISOLATION AND CHARACTERISATION OF ACTIVE CONSTITUENTS STIGMASTEROL FROM *CELOSIA SPICATA*FAMILY*AMARANTHACEAE*

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ABSTRACT

The focus of this present research is based on the analytical methodologies, which include the isolation of active ingredients from *Celosia Spicata* plant family*Amaranthaceae*. This study emphasises the isolation of active compounds using column chromatography and with the help of TLC, solvent system identified which help in the characterisation of isolated compound. Furthermore characterisation performed via UV-visible Spectroscopy, FTIR (Fourier Transform Infrared spectroscopy), NMR (Nuclear Magnetic Resonance), and Mass Spectrometry with respect to standard compound. AdditionallyI*n vitro*antibacterial activity of *Celosia Spicata* plant was assessed byWell Diffusion assay.Quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature.At the end result of all the analysis proven that the compound which was isolated from *Celosia Spicata* extract waspureStigma sterol.

Keywords: *Celosia Spicata* ,isolation, antibacterial activity,Well Diffusion assay

INTRODUCTION

Products of natural origins can be called ‘‘natural products.’’ Natural products include: (1) a complete creature (such as a plant, animal, or microorganism) that has not undergone any form of processing or treatment aside from a straightforward preservation process (such as drying), (2) a component of an organism (such as a plant's leaves or blossoms or a single animal organ), (3) a portion or extract of an organism, as well as exudates, and (4) pure compounds (e.g., alkaloids, coumarins, flavonoids, glycosides, lignans, steroids, sugars, terpenoids, etc.) isolated from plants, animals, or microorganisms (**Grabley et al.,1991**). However, in most cases the term natural products refers to secondary metabolites, small molecules (mol wt <2000amu) produced by an organism that are not strictly necessary for the survival of the organism. Products of overflow metabolism due to food deficiency, shunt metabolism created during idiophase, defence mechanism regulator molecules, etc. are examples of secondary metabolism concepts(**Dawson et al.,1992**). Natural products can come from any terrestrial or aquatic source, including plants, animals, or microbes (such as doxorubicin from Streptomyces peucetius or paclitaxel [Taxol] from Taxus brevifolia). About 40% of currently used contemporary medications were created from natural ingredients. More specifically, according to Cragg et al., 60-80% of antibacterial and anticancer medications came from natural sources, and 39% of the 520 new drugs that were approved between 1983 and 1994 were natural compounds or their derivatives (**Sidebottom, et al.,1992**). Herbal remedies for a variety of ailments are becoming more popular. One plant's components may be utilized to make a broad range of drugs, which are then employed in the

medical field. The use of herbal medicine is widespread around the world, in both developed and developing nations, for the prevention and treatment of a variety of health problems and illnesses, such as the common cold and flu (**World Health Organization; 2013**). Medical plants have been used for generations to cure and prevent a wide range of maladies, but some believe that they are dangerous to humans as well. In contrast, domesticated plant species, which have been grown by humans, rely on life control methods such as selection or cultivation. Wild plant species may flourish in both natural and man-made situations, thus it's possible that they are wild plant species (**Dias et al.,2012;Tova 2004**). Celosias belong to the amaranth family, Amaranthaceae**(Wang et al.,2006; Shanmugam et al.,2011).***Celosia Spicata* is a tall, upright plant with reddish purple foliage and eye-catching dark-pink, soft-pink terminal flower spikes that turn to white as they mature. It grows up to 81.3 cm in height and is considered a weed in some regions of the world, but eaten as a vegetable in Africa. *C. spicata* L. is known as a leafy vegetable in south western part of Nigeria (**Ogungbenle et al.,2015**), and is one of the most popular cut flowers for dried, everlasting floral arrangements. It occurs in tropical Africa and humid areas**(Dian 1994).** Its common names include wheat straw, Flamingo feather in English, Sufaid murghu in Hindi.

Materials and methods

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant by soxhlet extraction method

Coarsely powered flowersof *Celosia Spicata* was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 36 hrs using soxhlet apparatus**(Alara et al., 2019)**.

Formula;

$$\%\ yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x}100$$

Phytochemical screening of the extract

A variety of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were qualitatively analysed in the extract of *Celosia Spicata* (**Kokate *et al*.,2006**).

Quantitative Phytochemical estimation

Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measuredspectrophotometrically **(Tangco *et al.*, 2015).**

Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was determined using Aluminium chloride method **(Chang *et al.*, 2002)**. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight **(Parthasarathy *et al.*, 2009).**

Isolation-

Thin Layer Chromatography

Thin Layer Chromatography of *Celosia Spicata* extractwas carried out on TLC plates of silica gel 60 F_{254} pre coated with layer thickness of 0.2 mm using different solvent systems. Spots on TLC plates were visualised with spraying reagent: sulphuric acid solution, then in UV light. R_f values were calculated **(Kumar *et al.*, 2018).**

Column chromatography

Methanol extract was subjected to silica gel column chromatography for isolation of bioactive components from *Celosia Spicata* extract.Gradient elusion technique was followed for column chromatography. The column was eluted with n-hexane: Acetone (8:2) and number of elutes were collected.**(Srivastava*et al.*, 2021)**

Characterization-

UV-visible Spectroscopy

The isolated fraction of sample was diluted to 1:10 with the same solvent. The extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1800) and the characteristic peaks were detected and recorded **(Perkampus.*et al.*, 2013)**.

FT-IR

To establish the presence of the functional groups, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer.**(Lucien*et al.*, 2008).**

NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction to identify the structure of the compound present in the isolated fraction. ^{1}H NMR spectra of synthetic compounds were recorded on NMR Spectrometer (Bruker AV 500, at 500.130 MHz) **(Nayak *et al.*, 2015).**

Mass Spectroscopy

The mass spectrometer used for the identification of the molecular weight of the compound was Bruker Daltonik, Benchtop easy-to-use, high performance Electrospray Ionization Quadrupole time-of-flight LC MS spectrometer **(Wiley *et al.*, 1995).**

Anti-bacterial Activity

Well Diffusion Assay

The bacterial suspension (S. mutans) was standardized to 10 ^{8} CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate (**Mohammadi-Sichani *et al.*, 2012)**.100 µlof the sample was loaded. Two wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with samples. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 o C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm**.**

Table 1:Total phenolic and total flavonoid content *Celosia Spicata* extract

S. No.	Solvents→ Bioactive compound↓	Methanolic extract
***Celosia Spicata* extract**		
1.	Total phenol (Gallic acid equivalent (GAE) mg/100mg)	69
2.	Total flavonoid (Rutin equivalent (RE) mg/100mg)	47

Figure 1: TLC estimation of all collected fractions *of CSE* (M) after column chromatography & Std Sterol

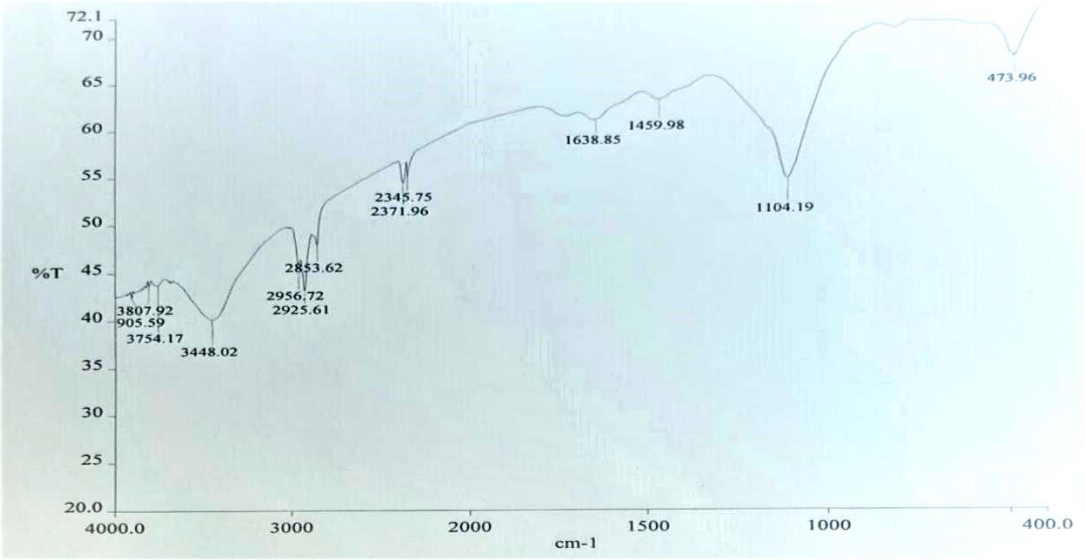

Figure 2 : IR spectra of the isolated compound (Fraction A) of ***Celosia Spicata* Flower (Methanol) extract**

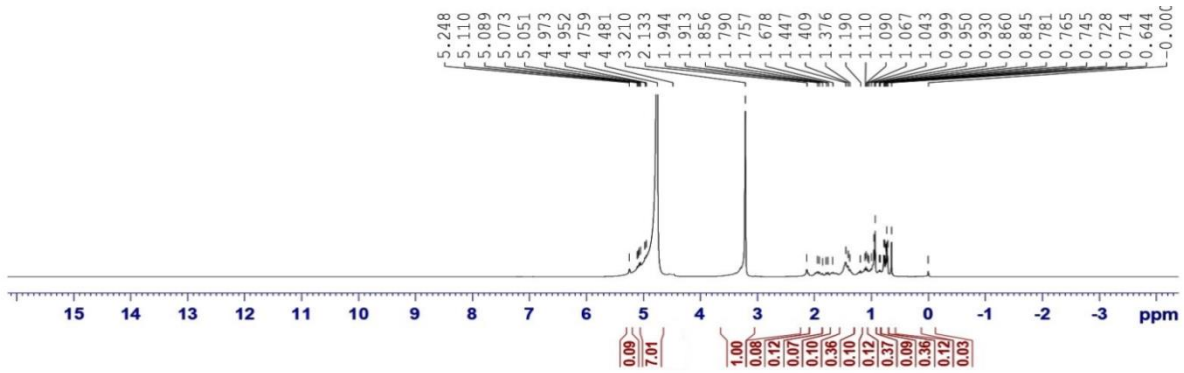

Figure 3 : ^{1}H NMR spectra of the isolated Fraction (Fraction A) of ***Celosia Spicata*** **Flower (Methanol) extract**

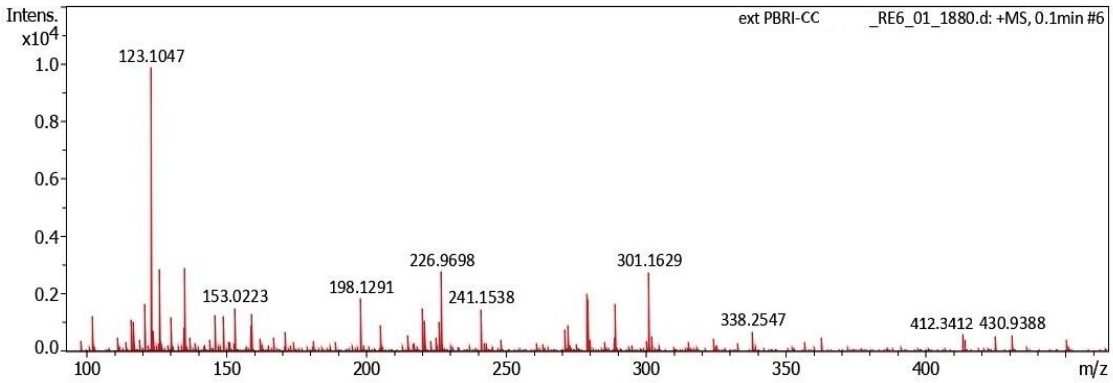

Figure 4: **Mass spectra of the isolated Fraction (Fraction A) of *Celosia Spicata* Flower(Methanol) extract**

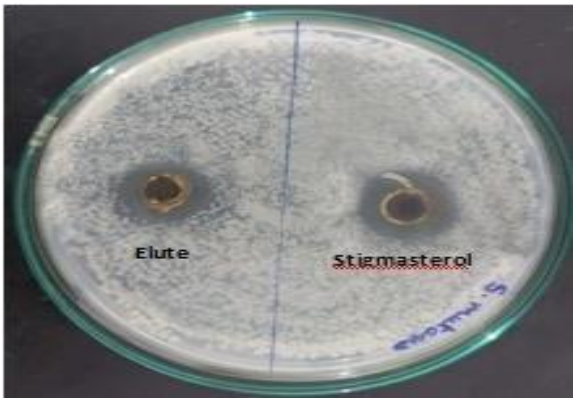

Figure 5 : Antibacterial activity of the isolated compound and standard stigma sterol

DISCUSSION

The present study revealed that a result of *Celosia Spicata* Flower(Methanol) extract contains Phytochemical constituents like flavonoids, glycosides, Carbohydrates, phenolis, Tannins, Alkaloids compounds by phytochemical investigation with respect to chemical tests and Preliminary TLC chromatographic techniques. In the Preliminary TLC for *Celosia Spicata* Flower(Methanol) extract in which the spots were visible in n-hexane: Acetone (8:2) mobile phase and Rf value of *CSE* (M)and Std. Sterol were found to be 0.44 and 0.44. So that n-hexane: Acetone (8:2) solvent was taken as mobile phase for column chromatography. Active constitutes are isolated from column chromatography with the mobile phase of n-Hexane: acetone (8:2) to obtained *CSE* (M)Fractions 01 (A), 02-04 (B = B1, B2, B3), 05-10

(C = C1, C2, C3, C4, C5, C6). Rf value Resulted after performing the TLC estimation is also done for the confirmation of active constituents in fraction A of *CSE* (M)by comparing with standard Sterol. In IR spectrum of isolated Fraction A of *CSE* (M) , a very intensely broad peak at 3448.02 cm^{-1} was observed for the O-H bond vibrations of hydroxyl group. The corresponding C=C vibrations was shown around 1638.85 cm^{-1} as weakly intense peak. The stretching and bending vibrations of methyl part were noticed by the intense peak 2925.61 cm^{-1}. The vibration of the methylene part was shown by the peak at 2853.62 cm^{-1} and the medium peak at 1459.98 cm^{-1}. The corresponding C-C vibration was shown as weak intense peak at 1104.19 cm^{-1}.In ^{1}H-NMR spectrum isolated fraction (Fraction A) of *Celosia Spicata* Flower (Methanol) extract, H-3 protons appeared at 3.21 ppm (tdd) and H-6 protons showed at 5.11 ppm (m). H-3 protons appeared at 5.24 (S) and 2.13 ppm (m). H-3 protons each also appeared at 1.94 ppm (s), 1.75 ppm (s), 1.19 ppm (s), 1.04 ppm (s), 0.95 ppm and 0.84 ppm (s).In Mass spectrum of isolated fraction (fraction A) of *Celosia Spicata* Flower (Methanol) extract showed molecular ion [M^{+}] peak at mlz 412.3412 which corresponds to the molecular formula $C_{29}H_{48}O$ according to their fragments. The antibacterial assay of formulation coded ESS-1 and EIS-1 (elute) were performed using the well diffusion method against S. mutans. Standard stigmasterol showed best zones of inhibition of 15±1 mm in diameter on the other hand elute showed best zones of inhibition of 11.33±1.527mm in diameter.

CONCLUSION

The phytochemical examination of the parts of Flower of *Celosia Spicata* belonging to the family *Amaranthaceae*was effectively carried out. From these physical, chemical and spectral evidences was confirmed as Stigmasterol in fraction A of *CSE* (M).

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