Section A -Research paper

Phytochemical characterization of *Curcuma aromatica (*rhizome)

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Abstract:
Phytoconstituents are bioactive compounds such as Alkaloids, phytosterols, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. determine the therapeutic value of a medicinal plant either individually or in combination. The current study has performed the phytochemical (phytosterol, alkaloid and flavonoid) isolation of *Curcuma aromatica* using Thin layer chromatography (TLC) and column chromatography and characterization was done using GS- MS, UV-Vis spectroscopy and FTIR analysis. In the results of TLC analysis, the presence of different phytosterols (lanosterol (0.96 R_f), β-sitosterol (0.88 R_f), stigmasterol (0.83 R_f) and Campesterol (0.28 R_f)), alkaloids (trigonelline-0.092 R_f), and flavonoids (luteolin (0.57 R_f), quercetin (0.77 R_f) and kaempferol (0.87 R_f) were identified. During the GC-MS analysis, a total of 11 phytosterols compounds, 7 alkaloids compounds, and 14 flavonoids bioactive compounds were identified in which the Campesterol (phytosterol), lupanine (alkaloid) and Kumatakenin (flavonoid) were identified as the novel compounds. The characterization of the isolated novel compounds was done using UV-Vis spectroscopy and FTIR analysis. The UV-Vis spectroscopy showed different wavelengths for phytosterols, alkaloids and flavonoids (205 nm, 264.59 nm, and 274 nm). Also, during the FTIR analysis, different functional groups of the present bioactive compounds were analyzed.

Keywords: *Curcuma aromatica,* phytosterol, alkaloids, flavonoids, GC-MS, TLC, FTIR, UV-Vis.

Introduction

Plant materials contain a high concentration of biologically active metabolites. Some of these plants' active secondary metabolites contain potential bioactive compounds of interest to the pharmaceutical industry. Plant-derived substances have recently intrigued the interest of researchers due to their potential applications as drugs, model compounds for drug synthesis, or intermediates for synthetic drugs (**Ncube *et al.*, 2008**). The World Health Organization (WHO) defined herbal preparations produced by extraction, fractionation, purification, concentration, or other physical or biological processes that may produce nutritional or medical compounds for immediate consumption or as a basis for herbal products. According to some authors, medicinal plants are plants that contain active ingredients that are used to cure disease or alleviate the pain (**WHO, 2001**).

Phytochemicals (Greek: phyton = plant) are chemical compounds found in plants that have either positive or negative health effects (**Silva *et al.*, 2017**). The richest bio reservoirs of various phytochemicals are medicinal plants used to treat various diseases and ailments. The phytochemical constituents of plants determine their medicinal properties (**Ezeonu *et al.*, 2016**). Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and other important phytochemicals are found in various parts of plants (**Sheel *et al.*, 2014**). Nature is a unique source of structures with high phytochemical diversity, including phenolics (45%), terpenoids and steroids (27%), and alkaloids (18%) (**Saxena *et al.*,2013**) Although these compounds appear to be non-essential to the plant that produces them, they play an important role in survival by mediating ecological interactions with competitors, protecting them from diseases, pollution, stress, UV rays, and contributing to the plant's colour, aroma, and flavour. Plant metabolites used to protect themselves from biotic and abiotic stresses have been transformed into medicines that people can use to treat a variety of diseases **(Njoku *et al*., 2009).**

Earlier, plant parts were used straight for treatment, but now the active principles are identified and isolated in pure form, as well as synthetically produced using advanced techniques (**Kocabus *et al.*, 2017)**. The chemical structures derived from these phytoconstituents can be used as models in the development of new synthetic drugs and the identification of phytoconstituents in plant material aids in predicting the plant's potential pharmacological activity **(Emran *et al.*, 2015).** Characterization and evaluation of plants and their phytoconstituents can uncover evidence to support therapeutic claims made by those plants for a variety of ailments (**Tiwari *et al.*, 2011**). Advanced techniques such as Gas Chromatography (GC), Liquid Chromatography (LC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), and others are very useful for both qualitative and quantitative detection of phytoconstituents (**Silva *et al.*, 2017**).

Curcuma species are widely used as a food additive and also in various medicinal purposes. The plant is a rich source of essential oil and is predominantly extracted from the rhizomes (**Albaqami *et al.*, 2022**). *Curcuma aromatica* Salisb. (Family: Zingiberaceae), also known as wild turmeric (Vana haridra) or yellow zedoary, is the second most commonly used curcumin species after common turmeric (*Curcuma longa* Linn.). The plant grows wild throughout India and is primarily cultivated in Kerala and West Bengal (**Shamim *et al.*, 2011**). It has a long history of use as an aromatic medicinal cosmetic and is also a promising therapeutic drug. *Curcuma aromatic* (CA) is a common Chinese herb used to treat diseases characterized by blood stasis and is thought to be a potent anticancer herb. In indigenous medicine, it is used for external applications on skin diseases, sprains, bruises, snake poison, and to improve complexion. It has a higher volatile content (4-8%) than *Curcuma longa,* and the chemical and aroma characteristics of the volatile oil of the two species are also different. Because of the presence of camphene, camphor, and a high boiling alcohol in CA's volatile oil, they can be easily distinguished using thin layer chromatography (TLC) or gas chromatography (GC) (**Pant *et al.*, 2013**) Present study focuses on phytochemical screening and characterization of

*Curcuma aromatica (*Rhizome) by using TLC, column chromatography and GC-MS, FTIR and UV-Vis.

MATERIALS AND METHODS

(A) Collection of Plant Material

The experimental plant material i.e. *Curcuma aromatica* (Salib.) rhizome parts was collected from the University of Rajasthan in Jaipur city of Rajasthan state, India. The obtained plant was further identified and confirmed at the Herbarium of the Department of Botany in the University of Rajasthan.

(B) Isolation of Phytochemical Compounds using Thin Layer Chromatography

(i) Phytosterol compounds

Dried powdered plant material was defatted in petroleum ether (60-80^{o}C) for 24 h on a water bath. Defatted material was air dried and hydrolyzed in 30% HCl (v/v) for 4 h. Each hydrolyzed sample was washed with distilled water till pH 7 and was dried later. The dried preparation was again extracted with benzene for 24 h. The extract was filtered and dried *in vacuo*. The crude extract was dissolved in benzene before chromatographic examination (Kaul and Staba, 1968). Glass plates coated with silica gels G were used. These plates were developed in air tight chromatographic chamber, saturated with a solvent mixture (Hexane: Acetone (8:2), Fazli and Hardman, 1968). These plates were air-dried and were sprayed with 50% sulphuric acid (Bennet and Heftmann, 1962) and heated at 110° C for 10 min in an oven. Color and R_f values of spots were compared with authentic sample applied simultaneously. Stigmasterol was used as a standard.

(ii) Alkaloid compounds

Powdered plant materials were taken in 100 mL Erlenmeyer flasks containing distilled water (50 ml/g) and 5 ml of 0.05 N sulphuric acid was added to it. Mixture was macerated for 3-4 h and boiled gently for 25 min. Heavy magnesium oxide (2.5 g/g) was added to the mixture and again boiled gently for 20 min. It was cooled at room temperature and an equal amount of distilled water was added to make up for loss of distilled water during boiling. Alcohol was added to remove the mucus. Mixture was filtered through Whatman filter paper (Kogan *et al.*, 1953). Filtrate was evaporated to dryness *in-vacuo,* reconstituted in distill water for further analysis. Extracted sample were dissolved in distilled water and applied on activated TLC plates, along with authentic samples of trigonelline. These plates were developed in pre-saturated chromatographic chamber containing solvent mixture of Butanol: Acetone: Water (4:1:5) of Johnson and Linn, 1953. Further plates were air dried at room temperature. Plates were sprayed with Dragendroff's reagent and heated at 10^{o}C for 10 min in an oven. Color and R_f values of spots were compared with authentic sample applied simultaneously.

(iii) Flavonoid compounds

The *Curcuma aromatica* (Salib.) rhizome parts were air-dried and powdered, separately. Each of these was extracted separately with 80% methanol on a water bath (Subramanian and Nagarajan, 1969) for 24 hrs. The methanol soluble fractions were filtered, concentrated *in* vacuo and aqueous fractions were fractioned by sequential extraction with petroleum ether (FrI), diethyl ether (FrII), and ethyl acetate (FrIII) separately. Each step was repeated thrice

for complete extraction, fraction I was discarded in each case because it contained fatty substances, whereas fractions II and fraction III were concentrated and used for determining flavonoids. Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid (10mL/g plant material for 2 h), filtered and the filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings, and concentrated *in vacuo.* Both fractions II and III were taken up in a small volume of ethanol (2-5ml) before the chromatographic examination. Thin glass plates (20x20 cm) were coated with Silica gel G (250mμ thick). The freshly prepared plates were air-dried at room temperature; thereafter these were kept at 100ºC in an oven for 30 minutes to activate and then cooled at room temperature. The freshly prepared and activated plates were used for analysis. Each of the extracts was co-chromatographed with authentic flavonoid as a marker (quercetin, luteolin, and kaempferol). These plates were developed in an air-tight chromatographic chamber saturated with the solvent mixture (Benzene: Acetic Acid: Water, 125:72:3, Wong and Francis, 1968). The developed plates were air-dried. The developed plates were sprayed with 5% $FeCl_3$, and kept in the I_2 chamber separately. The colored spots thus developed were noted and the R_f value of each spot was calculated.

(C) Isolation of Phytochemical Compounds using Column Chromatography

(i) Phytosterol compounds

The isolated phytosterol extract was subjected to column chromatography using a stationary phase silica gel 60 and as the mobile phase hexane was used. The fractions are concentrated on a rotary evaporator pressure reduced. The phytosterol isolates were identified using FTIR (Perkin Elmer Spectrophotometer), UV-vis and GC-MS (Agilent 6890).

(ii) Alkaloid compounds

The prepared alkaloid extract was subjected to column chromatography eluting with methanol. Fractions were collected and characterized by using FTIR (Perkin Elmer Spectrophotometer), UV-vis and GC-MS (Agilent 6890).

(iii) Flavonoid compounds

The isolated flavonoid extract was put into the packing column and then eluted with eluent. Elution was started with ethanol. The resulting eluate was collected in vials and identified using FTIR (Perkin Elmer Spectrophotometer), UV-vis and GC-MS (Agilent 6890).

(D) Identification of Phytochemical Compounds by Various Techniques

(i) Gas Chromatography and Mass Spectroscopy (GC-MS)

GC-MS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different compounds within a test sample. Bioactive compound namely phytosterol, alkaloid and flavonoid were identified by analyzing phytosterol, alkaloid and flavonoid extracts of *C. aromatica* rhizome parts. An Agilent 6890 GC with 5975 B mass spectrometric detector (MSD) was used in the scan mode (m/z 3940), low mass (m/z 45) and high mass (m/z 450) for extracts. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 1 µl was employed

(split ratio of 10:1). Injector temperature 250°C, Ion-source temperature 280°C was used. The oven temperature was programmed from 110°C (isothermal for 2 min.) with an increase of 10°C-200°C/min then 5°C-280°C/min ending with a 9 min. and isothermal at 280°C. Mass spectra was taken at 70 eV, a scan interval of 0.5 sec and fragments from 45 to 450 Da. Total GC running time was at 33.10 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was Turbo Mass Ver 5.2.0; screening for volatiles and semi volatiles compounds (Pasricha *et al.*, 2014).

ii) UV- Vis

Samples (1 mL) of the suspension were collected periodically to monitor the completion of bio-reduction in aqueous solution, followed by dilution of the samples with 2 ml of deionized water and subsequent scan in UV-visible (vis) spectra, between wave lengths of 200 to 700 nm in a spectrophotometer (Beckman - Model No. DU- 50, Fullerton, CA, USA), having a resolution of 1 nm. UV-vis spectra were recorded at intervals of 0 min, 15 min, 30 min, 45 min, 60 min and 24 h.

iii) FTIR Spectroscopic analysis

For FTIR spectrophotometer analysis, the phytochemical compounds were dissolved in benzene and alkaloid and flavonoid extracts were dissolved in ethanol and each extract were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The samples were diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

RESULTS AND DISCUSSION

(A) Isolation of Phytochemical Compounds

(i) Phytosterol compounds

Thin layer chromatography (TLC) is one of the most popular and simple chromatographic techniques used for the separation of compounds. TLC is being employed extensively in the standardization of herbal extracts **(Nikam *et al.*, 2012)**. Phytosterol is an unsaponifiable lipid fraction of plant-based foods and is a potential source of bioactive components (**Ryan *et al.*, 2007**). The initial screening of phytosterol compounds in *Curcuma aromatica* rhizome was performed by TLC method. In *Curcuma aromatica* rhizome solution lanosterol (0.96 R_f), β-sitosterol (0.88 R_f), stigmasterol (0.83 R_f) and Campesterol (0.28 R_f) phytosterol compounds were visualized in pink brown, purple brown, and grey colour (Table 1 and Figure 1). As reported by **Naumoska *et al.*, 2015,** three TLC methods were used for an initial screening of some common plant triterpenoids and phytosterols in cuticular wax extracts of different vegetables (zucchini, eggplant, tomato, red pepper, mangold, spinach, lettuce, white-colored radicchio di Castelfranco, raddicchio Leonardo, white cabbage, red cabbage and savoy cabbage) and the average R_f values with standard deviations (SD) calculated for each studied

compound from three HPTLC C18 RP plates developed in the normal developing chamber with ethyl acetate–acetonitrile (3:2, v/v) to a distance of 18 cm i.e., Stigmasterol 0.16 ± 0.02, Cycloartenol 0.28 ± 0.01, α -Amyrin 0.30 ± 0.02 , β -Amyrin 0.33 ± 0.01, Cycloartenol acetate 0.40 ± 0.01, Lupeol 0.42 ± 0.01, Friedelin 0.43 ± 0.01, Lupeol acetate 0.50 ± 0.01, Lupenone 0.57 ± 0.01. Likewise, **He *et al.*, 2018** study was to establish a green and highly efficient method to synthesize phytosterol linolenate for the first time by employing Bronsted acidic ionic liquid (IL) as a catalyst in order to improve its oil solubility. In the result of TLC analysis, the R_f values of phytosterols was 0.04–0.13.

Table 1: Chromatographic characteristics of Phytosterol compounds isolated from *Curcuma aromatica* rhizome

Isolated compounds	**R_f value**	**Colour after spray with R**
	S	
Lanosterol	0.96	PK-BN
β-sitosterol	0.88	PU-BN
Stigmasterol	0.83	GY
Campesterol	0.28	GY
Standard (Stigmasterol)	0.81	GY

Abbreviations: S- Hexane : acetone (8 : 2), R- 50% H_2SO_4, BN- Brown, PK- Pink, GY- Gray, PU – Purple

Figure 1: TLC of Phytosterol compounds isolated from *Curcuma aromatica* rhizome

(ii) Alkaloid compounds

The term "alkaloid" is applied to nitrogen-containing compounds, produced primarily in higher plants but also in lower organisms and in some animals, that have significant pharmacological activity (**Waller *et al.*, 2012**). As shown in table 2, alkaloid compound i.e., trigonelline was identified in brick red colour from *C. aromatica* rhizome at 0.092 R_f value. The mobile phase Butanol: Acetone: Water (4:1:5) gave significant result of separation of compounds from the sample (Figure 2). Result of the study conducted by **Kristani *et al.*, 2015** on *Atrocarpus communis* indicated that alkaloid was detected in chloroform extracts of leaves with R_f score 0.76 and methanol extracts of leaves with R_f 0.8. This showed that the leaves of bread tree contained polar and semipolar or nonpolar alkaloid. In the phytochemical screening and TLC profiling of various extracts of *Reinwardtia indica* by **Sonam *et al.*, 2017** in acetone extract alkaloid were detected at R_f 0.56.

Table 2: Chromatographic characteristics of Alkaloid compounds isolated from *Curcuma aromatica* rhizome

Isolated compounds	**R_f value**	**Colour after spray with R**
	S	
Trigonelline	0.092	BK-RD

Abbreviations: S- Butanol: Acetone: Water (4:1:5), R- Dragendroff's reagent, BK- Brick, RD- Red

Figure 2: TLC of Alkaloid compounds isolated from *Curcuma aromatica* rhizome

(iii) Flavonoid compounds

Flavonoids are the low molecular weight polyphenolic secondary metabolic compounds, universally distributed in green plant kingdom, located in cell vacuoles. Flavonoids play a variety of biological activities in plants, animals, and bacteria. In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for color, aroma of flowers, fruit to attract pollinators consequently fruit dispersion; help in seed, spore germination, growth and development of seedling (**Samanta *et al.*, 2011**). In chromatographic characterization, luteolin (0.57 R_f value), quercetin (0.77 R_f value) and kaempferol (0.87 R_f value) with yellow-brown spot after spray were the compounds identified from flavonoid extract of *Curcuma aromatica* as shown in Figure 3 and Table 3 with quercetin taken as standard. As per the study of **Kristanti and Tunjung, 2015** the qualitative estimation of *Artocarpus communis* leaf revealed Kaempferol with 0.89 R_f value with Rutin taken as standard. In the study conducted by **Dewatisari *et al.*, 2021** on the potency of *Sansevieria trifasciata* and *S. cylindrica* leaves extracts, flavonoid was detected with R_f 0.69 and R_f 0.93. Also, in the study of **Jadhav *et al.*, 2021** reported that TLC plate of *Asplenium indicum* showed R_f value range from 0.44 to 0.70 having yellowish green colour which indicates the presence of flavonoids. Column fractions from 110 to 119 with ethyl acetate:

ethanol (80:20) in the TLC mobile phase solvent ratio of chloroform : methanol (1:1) showed R_f value of 0.46 equal to that of standard quercetin as reported by **Sambandam e*t al.*, 2016**

Table 3: Chromatographic characteristics of Flavonoid compounds isolated from *Curcuma aromatica* rhizome

Isolated compounds	**R_f value**	**Colour of Spots**	
	S	**Day light**	**I_2 Vapour**
Luteolin	0.57	GN-YW	YW-BN
Kaempferol	0.87	GN-YW	YW-BN
Quercetin	0.77	GN-YW	YW-BN
Standard (Quercetin)	0.77	GN-YW	YW-BN

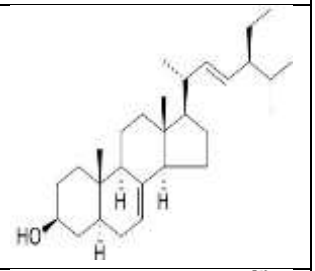
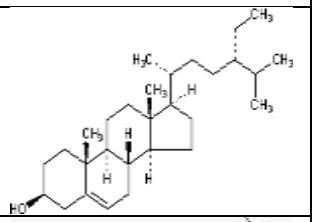
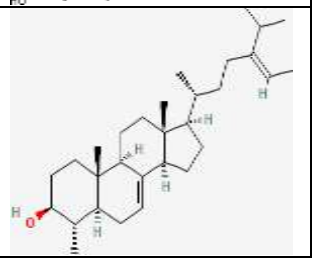
Abbreviations: S– Benzene: acetic acid: water (125: 72: 3), YW – Yellow, BN – Brown, GN – Green

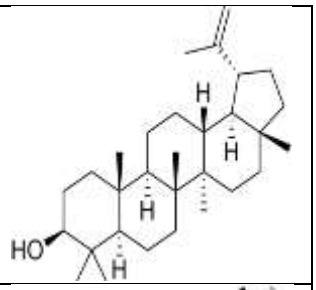
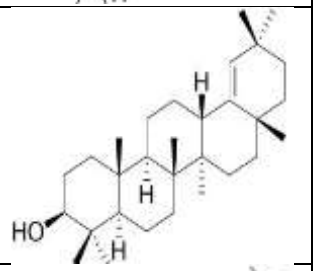
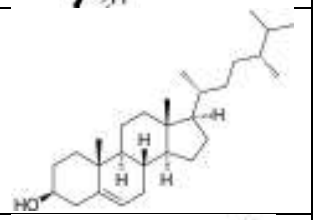
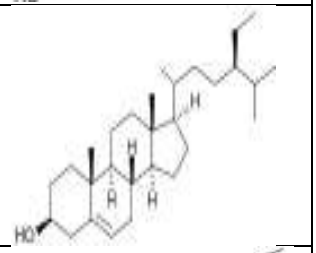
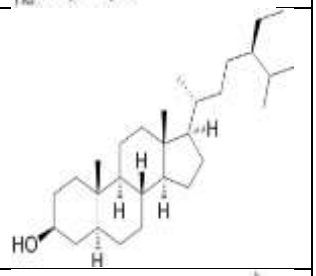
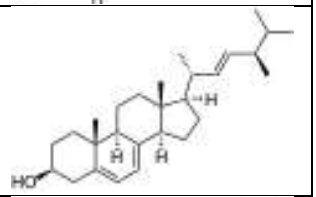
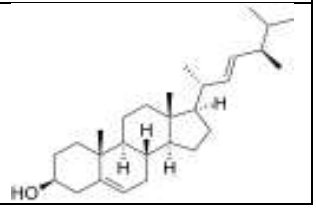
Figure 3: TLC of Flavonoid compounds isolated from *Curcuma aromatica* rhizome

(B) Identification of Phytochemical Compounds by Various Techniques
(i) Phytosterol compounds

The GC-MS characterization of phytosterol fraction of *Curcuma aromatica* rhizome revealed almost 11 compounds with significant concentration. The maximum level of phytosterol was observed of beta-sitosterol with 12.49% and minimum amount was campesterol i.e., 0.12%. Alpha-spinasterol, γ–sitosterol, α1-sitosterol, Lupeol, Stigmastanol, Ergosterol, Brassicasterol was present 0.19%, 1.27%, 1.09%, 0.47%, 0.31%, 3.91% and 4.27% (Table 8). Six cularine alkaloids, cularicine, *O*-methylcularicine, celtisine, cularidine, cularine and celtine; three isocularine alkaloids, sarcophylline, sarcocapnine and sarcocapnidine; and five non-cularine alkaloids, glaucine, protopine, ribasine, dihydrosanguinarine and chelidonine, were identified and quantified by GC-MS in nine taxa of the genus *Sarcocapnos* (Fumariaceae) by **Suau *et al.*, 2005** and the GC-MS analysis of non-polar fraction from *Ficus carica* L. leaves the phytosterols (18% of TIC) - stigmasterol, β-sitosterol, lanosterol and cycloartenol were analyzed by **Ivanov *et al.*, 2018.** The further characterization of fraction was done by UV-vis and FTIR analysis

Table 4: GC-MS analysis of phytosterol fraction of *Curcuma aromatica* rhizome

S. NO.	Name of compound & Molecular Formulae	Area %	Structure of compound
1	Alpha-spinasterol IUPAC- (3β,5α,22E)-Stigmasta-7,22-dien-3-ol $C_{29}H_{48}O$, MW 413,	0.19	
2	γ–sitosterol IUPAC- (24S)-stigmast-5-en-3β-ol) $C_{29}H_{50}O$, MW 415	1.27	
3	α1-sitosterol IUPAC- 4α-methylstigmasta-7,24(28)Z-dien-3β-ol $C_{30}H_{50}O$, MW 427,	1.09	

4	Lupeol IUPAC- lup-20(29)-en-β-ol $C_{30}H_{50}O$, MW 427,	0.47	
5	Germanicol IUPAC- Olean-18-en-3-ol $C_{30}H_{50}O$, MW 427,	0.19	
6	Campesterol IUPAC- 3β-ergost-5-en-3-ol $C_{28}H_{48}O$, MW 401, RT 20.588	0.12	
7	beta-sitosterol IUPAC- 3β-stigmast-5-en-3ol $C_{29}H_{50}O$, MW 415,	12.49	
8	Stigmastanol IUPAC- 5α-Stigmastan-3β-ol $C_{29}H_{52}O$, MW 417,	0.31	
10	Ergosterol IUPAC- (22E)-Ergosta-5,7,22-trien-3β-ol $C_{28}H_{44}O$, MW 397,	3.91	
11	Brassicasterol IUPAC- 3β-ergosta-5,22-dien-3-ol $C_{28}H_{50}O$, MW 399,	4.27	

Under UV-visible spectroscopy the wavelength of phytosterol fractionated from *Curcuma aromatica* rhizome was observed at 205 nm. The range of UV-vis spectrum was 400-4000 nm (Figure 4). An analytical methodology by UV-Vis spectrophotometry was developed by

Araujo *et al.*, 2013 and validated to quantify phytosterols in the roots of *Acanthospermum hispidum* and their extracts. The analytical procedure was validated at 625 nm showing linearity, repeatability, intermediate precision, accuracy and robustness whereas in the study of **Joseph *et al.*,** the phytosterol constituents from *Andrographis paniculata* UV spectrum showed at 202 nm.

FTIR spectra of phytosterol fraction of *Curcuma aromatica* rhizome showed a broad absorption band (3336 cm^{-1}) in the range of 3000-3500 cm^{-1} that indicating hydroxy group. A small speak at 1625, 1348.51 and 1220 cm^{-1} bands observed from phytosterol fraction of *Curcuma aromatica* rhizome indicated hydroxy group, aryl-substituted C=C, OH in-plane bend and aromatic C-H in-plane bend as functional groups (Figure 5). In the FT-IR spectrum of phytosterols by **He *et al.*, 2018** the medium peak at 3420 cm^{-1} corresponded to the stretching vibration of hydroxyl group. The peaks at 2957 cm^{-1} and 2863 cm^{-1} were the asymmetrical and symmetrical stretching vibration of C-H in -CH3 group, respectively. The medium peak at 1375 cm^{-1} was the bending vibration of C-H in -CH3 group. The medium peak at 1462 cm^{-1} was the bending stretching vibration of C-H in -CH2 group. The weak peak at 1640 cm^{-1} was the absorption signal of C=C. The strong peak at 1052 cm^{-1} was the characteristic absorption signal of C-O. In the report of **Joseph *et al.*,** 2016 the phytosterol constituents from *Andrographis Paniculata* on subjection to IR spectroscopic analysis, the observed absorption bands are appeared at 3421 cm^{-1} (OH), 2958 cm^{-1} (CH3), 2850 cm^{-1} (CH2), 1460 cm^{-1}, 1053 cm^{-1} (C-O). The compound was identified as stigmast-5-en-3b-ol (b-sitosterol) by comparison of its IR, melting point, mixed melting point.

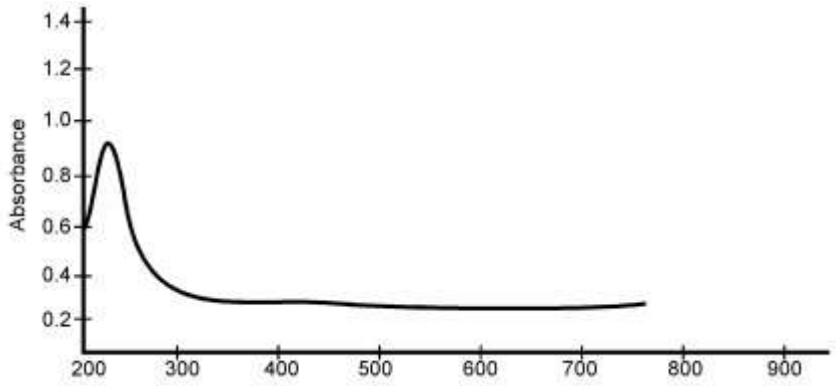


Figure 4: UV-Vis spectrum of phytosterol fraction of *Curcuma aromatica* rhizome

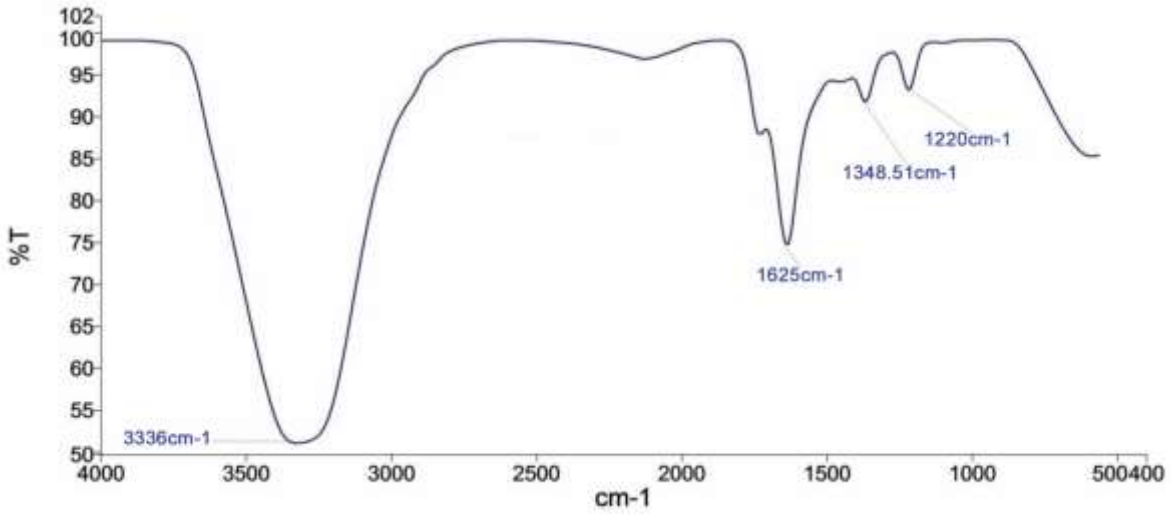


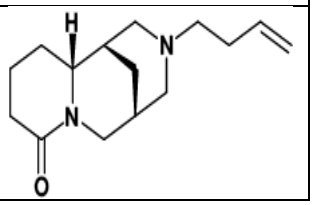
Figure 5: FTIR analysis of phytosterol fraction of *Curcuma aromatica* rhizome

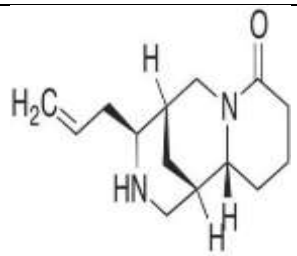
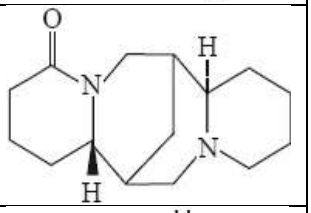
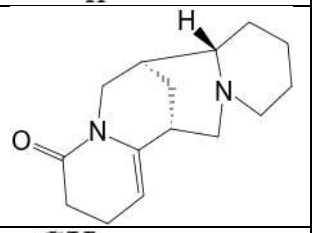
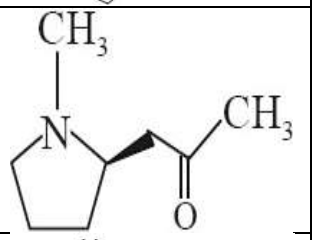
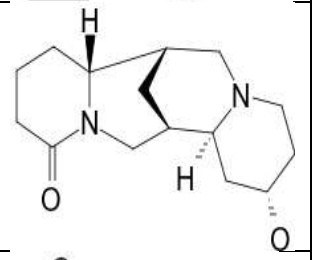
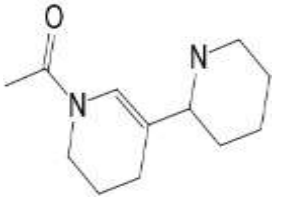
By comparing the results of GC-MS, UV-vis and spectra of IR and on the basis of literature available, the compound fractionated from *Curcuma aromatica* rhizome was Campesterol that showed 20.58 retention times which might be novel compound.

(ii) Alkaloid compounds

The GC-MS profile of alkaloid compound isolated from *Curcuma aromatica* rhizome fraction reported seven compounds from which lupanine was present in higher 2.44% amount other Tetrahydrorhombifoline, Angustifoline, 5,6-Dehydrolupanine, Hygrine, 13alpha-Hydroxylupanine and Ammodendrine was with 0.98%, 1.81%, 1.99%, 0.28%, 0.61% and 1.23% concentration, respectively (Table 5). Twenty-nine alkaloids from the extracts of *Dhatura stramonium* (Bulgarian origin) have been detected in the research of **Philipov *et al.*, 2002** by GC-MS including 3-Tygloyloxytropane, 3-Acetoxy-6-hydroxytropane, 3-Tygloyloxy-6-hydroxytropane etc. The presence of hygrine, physoperuvine, tropine, 3$-acetoxytropane, and two N-methylpyrrolidinylhygrine isomers, 3a-tigloyloxytropane and cuscohy- grine was detected in different organs of *Physalis peruviana* L. plants by GC-MS as reported by **Kubwabo *et al.*, 1993.**

Table 5: GC-MS analysis of alkaloid fraction of *Curcuma aromatica* rhizome

S. NO.	Name of compound & Molecular Formulae	Area %	Structure of compound
1	Tetrahydrorhombifoline IUPAC- (1*S*,2*R*,9*R*)-11-but-3-enyl-7,11-diazatricyclotridecan-6-one $C_{15}H_{24}N_2O$, MW 248	0.98	

2	Angustifoline IUPAC-(1*S*,2*R*,9*S*,10*S*)-10-prop-2-enyl-7,11-diazatricyclo tridecan-6-one $C_{14}H_{22}N_2O$, MW 234	1.81	
3	Lupanine IUPAC-(1*S*,2*R*,9*S*,10*S*)-7,15-diazatetracyclo heptadecan-6-one $C_{15}H_{24}N_2O$, MW 248,	2.44	
4	5,6-Dehydrolupanine IUPAC- 7,15-diazatetracyclo heptadec-2-en-6-one $C_{15}H_{22}N_2O$, MW 246	1.99	
5	Hygrine IUPAC- 1-[(2R)-1-Methylpyrrolidin-2-yl]propan-2-one $C_8H_{15}NO$, MW 141	0.28	
6	13alpha-Hydroxylupanine IUPAC-(1S,2R,9S,10S,12S)-12-hydroxy-7,15-diazatetracyclo heptadecan-6-one $C_{15}H_{24}N_2O_2$, MW 264	0.61	
7	Ammodendrine IUPAC- 1-[5-[(2*R*)-piperidin-2-yl]-3,4-dihydro-2*H*-pyridin-1-yl]ethanone $C_{12}H_{20}N_2O$, MW 208	1.23	

Under UV-Vis analysis (Figure 6) alkaloid compound isolated from *Curcuma aromatica* rhizome fraction showed a peak at 264.59 nm. In the study of **Costa *et al.*, 2018** the experimental spectrum of alkaloid showed bands at 222, 291, 300 and 391 nm in UV-vis that were assigned to the sum of the n/p* and p/p* transitions characteristic of b -carboline alkaloids. The qualitative UV-Vis spectrum profile of ethanol extract of *Sarcostemma brevistigma* was reported by **Dhivya *et al.*, 2017** in which the UV-Vis profile showed different peaks ranging from 200-1044 nm with different absorption respectively. UV-Vis profile showed the peaks at 254.00 and 680.00 nm for flavonoid.

In the FTIR analysis of *C. aromatica* rhizome alkaloid fraction spectra, three peak of functional groups were present in which two sharp peak of ketone group and methyl C-H asym./sym. bend was at 1735.11 cm^{-1} and 1370.31 cm^{-1}. One narrow peak was at 3460.20 cm^{-}

[1], indicating >N-H stretch. **Serdaroğlu *et al.*, 2021** showed the N–H single bond stretching appeared peak at 3359 cm^{-1} was assigned as a pure mode (100%) at 3560 cm^{-1} with IR. In addition, the observed peak at 1462 cm^{-1} related to the bond distortion was determined as the ipb HNC mode (69% PED) at 1437 cm^{-1}. In **2017, Dhivya *et al.*,** carried out their study to characterize the bioactive constituents present in aerial parts of ethanolic extract of *Sarcostemma brevistigma* using FTIR. FTIR spectra showed the peak at 3418.85 cm-1 for N-H group. The FTIR spectra had amply evidenced the occurrence of OH group together with the Terpenoids, and Phenol. The FT-IR spectrum showed the presence of an amine (N-H), alkyl (C-H), nitrile (C≡N), halo formyl (C=O), alkenyl (C=C), hydroxyl (O-H), haloalkane (C-F), nitrates and carbonate compounds.

On the basis of result of GC-MS, UV-vis, FTIR spectra and reported literatures, alkaloid compound was identified as lupanine, from *Curcuma aromatica* rhizome fraction. The molecular formula and weight of this compound was $C_{15}H_{24}N_2O$, MW 248. This compound is not reported in earlier so might be known a new compound.

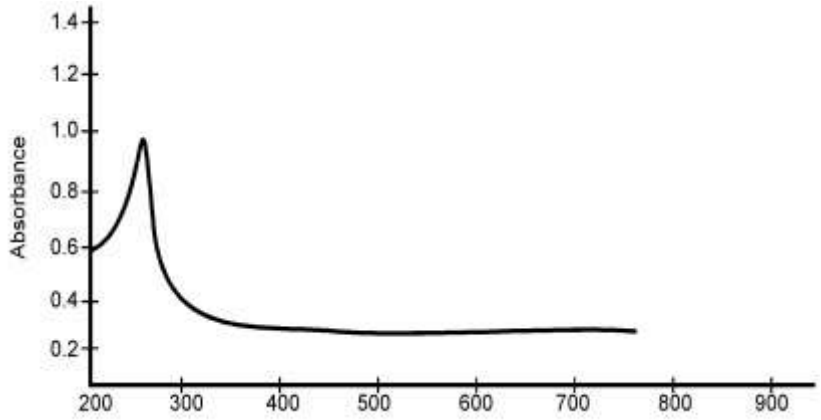


Figure 6: UV-Vis spectrum of alkaloid fraction *Curcuma aromatica* rhizome

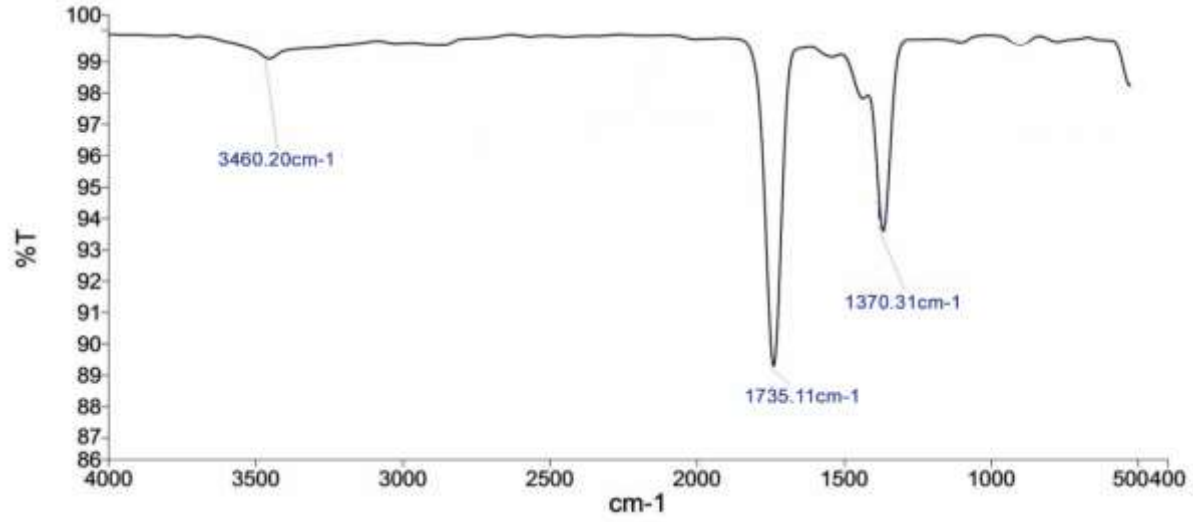
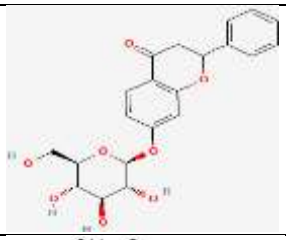
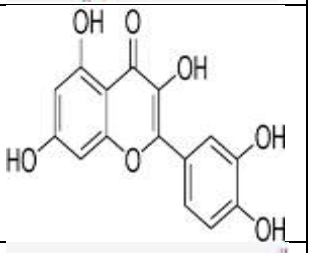
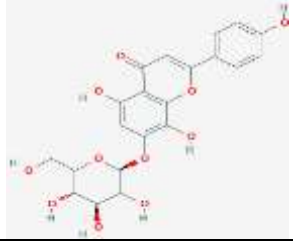


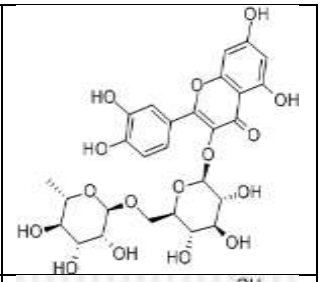
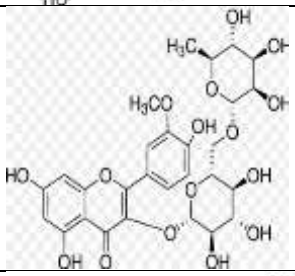
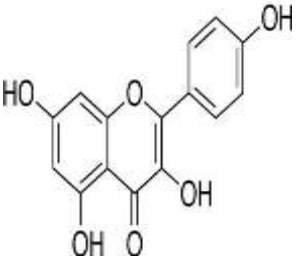
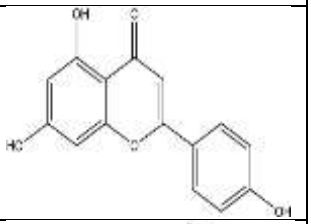
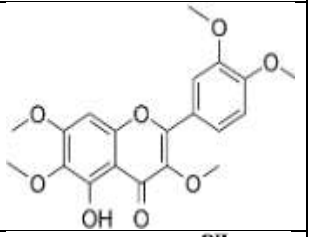
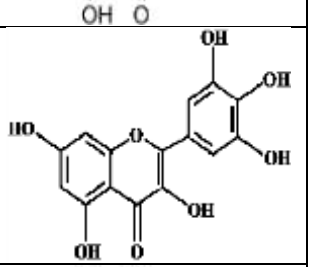
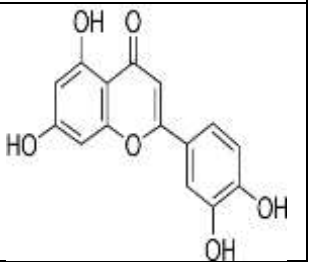
Figure 7: FTIR analysis of alkaloid fraction *Curcuma aromatica* rhizome

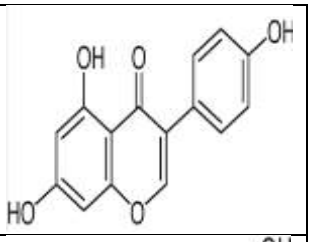
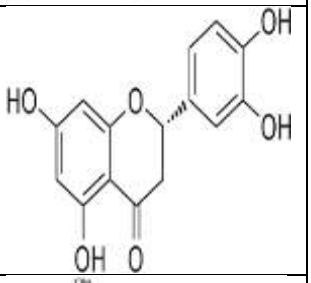
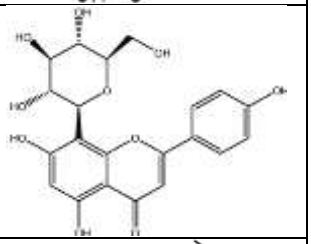
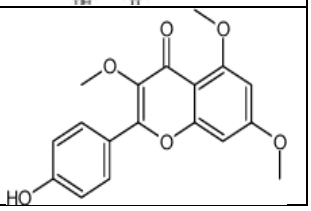
(iii) Flavonoid compounds

A total of 14 bioactive compounds were determined from the GC-MS profile of flavonoid fraction of *Curcuma aromatica* rhizome, in which Kumatakenin was detected as new compound with 0.28% concentration. 1.67% Flavanone 7-O-glucoside, 4.55% Quercetin, 0.99% Isoscutellarein 7-O-glucoside, 0.28% Rutin, 0.19% Quercetin 3-O-pentoside, 0.46% Kaempferol, 2.39% Apigenin, 1.23% Artemetin, 0.51% Myricetin, 0.97% Luteolin, 0.34% Genistein, 1.44% Eriodictyol, and 0.67% Vitexin was found and mentioned in Table 6. In the study of **Farag *et al.*, 2014,** GS-MS profile interpretation allowed for the identification of 10 flavonoid peaks. Detected flavonoids were di and tri-flavonol glycosides of quercetin and kaempferol based on their masses and UV spectral data, with kaempferol derivatives being more abundant as reported in *Nigella sativa.* Likewise, the hexane extract was analyzed by GC-MS technique in the study of **Keskes *et al.*, 2017** within *Juniperus phoenicea* leaves which allowed the identification of 32 compounds. Three flavonoids were identified in these fractions using HPLC-MS analysis: Quercetin 3-O-glucoside, isoscutellarein 7-O-pentoside and quercetin 3-O-pentoside.

Table 6: GC-MS analysis of flavonoid fraction of *Curcuma aromatica* rhizome

S. NO.	Name of compound & Molecular Formulae	Area %	Structure of compound
1	Flavanone 7-O-glucoside IUPAC $C_{21}H_{22}O_8$, MW 402	1.67	
2	Quercetin IUPAC- 3,3′,4′,5,7-Pentahydroxyflavone $C_{15}H_{10}O_7$, MW 302	4.55	
3	Isoscutellarein 7-O-glucoside $C_{21}H_{20}O_{11}$, MW 449	0.99	

4	Rutin IUPAC-3′,4′,5,7-Tetrahydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavone $C_{27}H_{30}O_{16}$, MW 611	0.28	
5	Quercetin 3-O-pentoside $C_{25}H_{26}O_{15}$, MW 567	0.19	
6	Kaempferol IUPAC-3,4′,5,7-Tetrahydroxyflavone $C_{15}H_{10}O_6$, MW 287	0.46	
7	Apigenin IUPAC- 4′,5,7-Trihydroxyflavone $C_{15}H_{10}O_5$, MW 271	2.39	
8	Artemetin IUPAC-5-hydroxy-3,6,7,3’,4’-pentamethoxy flavones $C_{20}H_{20}O_8$, MW 389	1.23	
9	Myricetin IUPAC-3,3′,4′,5,5′,7-Hexahydroxyflavone $C_{15}H_{10}O_8$, MW 319	0.51	
10	Luteolin IUPAC-3′,4′,5,7-Tetrahydroxyflavone $C_{15}H_{10}O_6$, MW 287	0.97	

11	Genistein IUPAC- 4′,5,7-Trihydroxyisoflavone $C_{15}H_{10}O_5$, MW 271	0.34	
12	Eriodictyol IUPAC- (2S)-3′,4′,5,7-Tetrahydroxyflavan-4-one $C_{15}H_{12}O_6$, MW 288	1.44	
13	Vitexin IUPAC-8-(β-D-Glucopyranosyl)-4′,5,7-trihydroxyflavone $C_{21}H_{20}O_{10}$, MW 432	0.67	
14	Kumatakenin IUPAC-5-Hydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy-4H-1-benzopyran-4-one $C_{17}H_{14}O_6$, MW 314	0.28	

The spectrum of UV-Vis of flavonoid fraction (Figure 8) revealed a peak of compound fractionated from *Curcuma aromatica* rhizome at 274nm. As reported by **Patle *et al*., 2020** in *Dillenia pentagyna* different absorption bands obtained in the UV–Vis region corresponded to the presence of quercetin (flavonoid) showed two absorption bands at 250 nm and 370 nm. The flavonoid extracts showed maximum absorption at the wavelength of 500 nm in visible range in *Portulaca oleracea* UV-Vis spectrophotometry as reported by **Zhu *et al*., 2010**

The IR spectrum denoted several absorption bands of functional groups of compounds fractionated from *Curcuma aromatica* rhizome. A total of four peak: two narrow, one broad and one sharp peak was observed from *C. aromatica* rhizome flavonoid fraction as shown in Figure 9. The absorption band at 1350 cm^{-1} indicated OH in-plane bend, 1231.44 cm^{-1} for aryl-O stretch, 1615 cm^{-1} for C=C-C aromatic ring stretch and 3346 cm^{-1} for OH stretch. As reported by **Sambandam *et al*., 2016 t**he broad absorption peak at around 3290 cm-1 was assigned to the OH stretching vibration of phenol. C=O aryl ketonic stretching vibrations are observed at 1668 cm^{-1}. The absorption peaks positioned at 1612 cm^{-1}, 1516 cm^{-1} and 1429 cm^{-1} are assigned to the C---C, C=O and C=C aromatic stretching vibrations respectively. C-O stretching vibrations of aryl ether and phenols were observed at 1240 cm^{-1} and 1210 cm^{-1} respectively. C-CO-C stretching and bending vibrations of ketones were observed at 1163 cm^{-1} which confirms that the isolated compound is flavonoid quercetin. **Patle *et al*., 2020** reported an ultrasonic-assisted extraction (UAE) of phytochemicals from bark, leaves, sepals,

fruits, and seeds of *Dillenia pentagyna* (Roxb) using different organic solvents such as chloroform, ethanol, and n-hexane. The FTIR spectrum of gallic acid, quercetin, rutin, and tannic acid in the frequency range of 4000–400 cm^{-1} was obtained to identify the characteristic absorption peaks corresponding to stretching vibrations of different functional groups. In quercetin, the peak obtained in the range of 3398–3314 cm^{-1} represented the O=H stretching vibration due to the intra-molecular hydrogen bonding. The prominent band observed at 1663 and 1606 cm^{-1} assigned to carbonyl C-O stretching vibration. The band obtained at 1520–1500 cm^{-1} assigned for NO_2 bending vibration, the peak at 1449–1400 cm^{-1} for C=O, the band at 1260–1200 cm^{-1} allocated to C–O–C of ester for quercetin compound. The prominent peak at 1164–1100 cm^{-1} indicated stretching vibration of the C-O-C group and the band between 819 and 800 cm^{-1}found for plane bending of = C–H.

Kumatakenin (RT 28.00) was determined from *Curcuma aromatica* rhizome flavonoid fraction by comparing the result of GC-MS, UV-vis and FTIR spectra. The several characterizations also helpful to known compound from fraction. The molecular formula of Kumatakenin was $C_{17}H_{14}O_6$, MW 314.

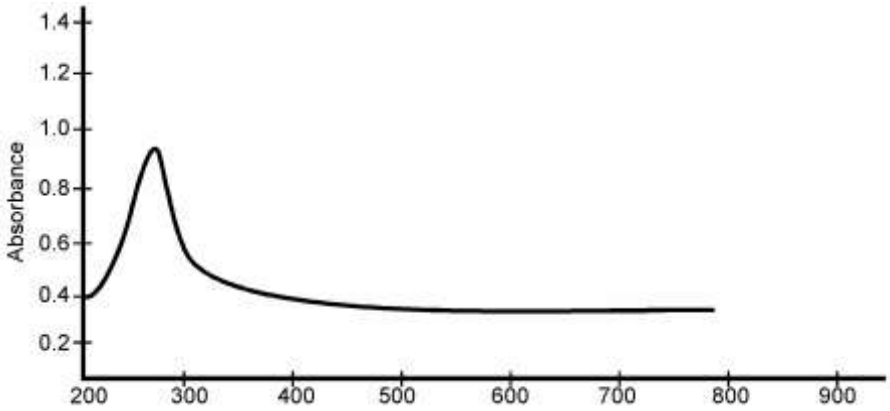


Figure 8: UV-Vis spectrum of flavonoid fraction of *Curcuma aromatica* rhizome

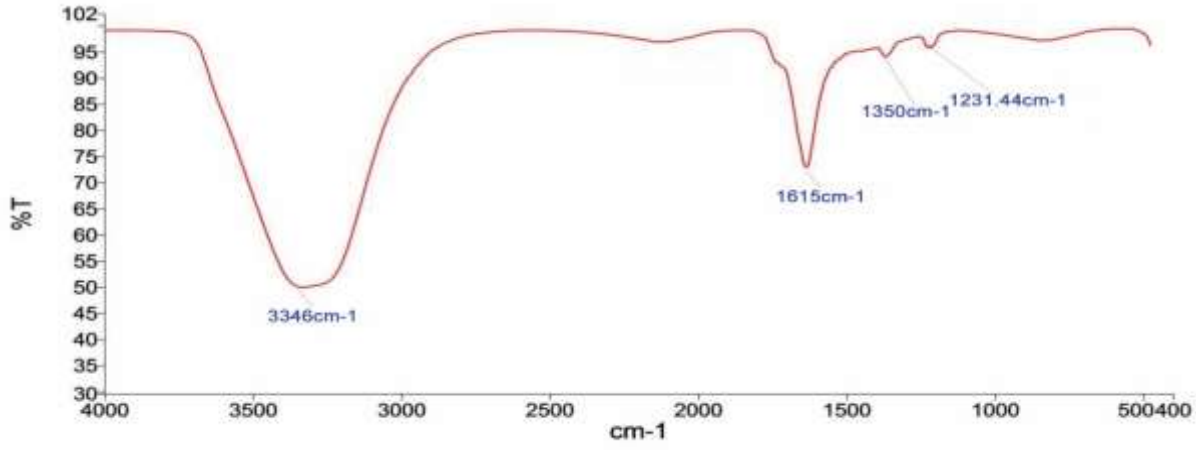


Figure 9: FTIR analysis of flavonoid fraction of *Curcuma aromatica* rhizome

Conclusion

In the present research, *Curcuma aromatica* which is a medicinal plant was used to study the presence of wide range of biological active compounds like phytosterol, flavonoids and alkaloids which can be exploited and well understood using modern technologies like GC-MS, FTIR and UV-Vis. As a result, a total of 11 phytosterols compounds, 7 alkaloids compounds, and 14 flavonoids bioactive compounds were identified in which the campesterol (phytosterol), lupanine (alkaloid) and kumatakenin (flavonoid) were identified as the novel compounds. The characterization of these novel compounds using UV-Vis spectroscopy and FTIR gave significant results. It can be concluded that the presence of these novel compounds in *C. aromatica* provides the medicinal property to the plant and needs a wide area of research into the detailed pharmacological actions. To increase the use of plants as the cheapest source of raw material in medicinal industry, phytochemical analysis also needs more research efforts.

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