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Antifungal metabolite from *Muntingia calabura* root against early leaf blight of tomato

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The aim of the present study is to determine the *in vitro* antimicrobial activity of various extracts and fractions of *Muntingia calabura* (Elaeocarpaceae) root against a selected panel of microorganisms. Antifungal activity of different solvent extracts of *M. calabura* L. root, tested against *Alternaria solani*, *Fusarium oxysporum* f.sp. *lycopersici, Pythium* species, *Phytophthora* species, *Aspergillus niger, Colletotrichum* species and *Rhizoctonia solani*, was evaluated by agar well diffusion assay. The chromatographic fractionation of the extract resulted in the isolation of antifungal metabolite stigmasterol. The structure of the stigmasterol was confirmed using GC-MS, IR and NMR spectroscopic characterization. The stigmasterol had a potent antifungal activity with a minimum inhibitory concentration of 1 mg/ml against *A. solani*. Stigmasterol was subjected to docking studies carried out against fungal elicitor cryptogein. A better docking score of 12.59 with glide energy -42.56 was obtained for the complex fungal elicitor cryptogein. The interaction was done in chain A Tyr 47 [O – H…O] residue with a distance of 2.7 A°.

Key words: *Muntingia calabura*, minimum inhibitory concentration, phytopathogens, cryptogein elicitor, stigmasterol.

INTRODUCTION

Plant being a major source of natural therapeutic remedies, has been used in various part of the world to treat infectious diseases (Vahidi et al., 2002). Recent focus of research for new source of safer and more effective antibacterial agents has been shifted towards natural products of plant sources (Souza et al., 2002; Nitta et al., 2002). Higher plants, which are able to produce photosynthesis, produce hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991). It is believed that these compounds have an important ecological role. These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Sarac and Ugur, 2007). There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of those extracts that yielded active principles (Rabe and Van Staden, 2000; Palombo and Semple, 2001; Portillo et al., 2001; Srinivasan et al., 2001; El-Seedi et al., 2002; Zgoda-Pols et al., 2002).

Muntingia calabura L. (Kerukup siam), also known locally as Jamaica cherry, is a plant of the family Elaeocarpaceae (Morton, 1987). It is native to the American continent and is widely cultivated in warm

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areas of Asian region. The leaves, barks and flowers are believed to possess medicinal value as reported in Peru folklore medicinal uses. The roots have been employed as an emmenogogue in Vietnam and as an abortifacient in Malaysia (Chin, 1989). Various parts of this tree have several documented medicinal uses in both Southeast Asia and tropical America (Nshimo et al., 1993). The roots of *M. calabura* L. (Elaeocarpaceae) were investigated as part of a continuing project to discover novel antineoplastic agents of plant origin (Kaneda et al., 1991). From a cytotoxic Et_2O-soluble extract of *M. calabura* roots, twelve new flavonoids were isolated, constituting seven flavans, three flavones and two biflavans. Scientifically, several types of flavonoids and flavones have been isolated and identified from this plant (Su et al., 2003; Chen et al., 2005). Therefore, the main objective of this study is to search for the active fraction with strong antimicrobial activity which could serve as a good candidate for the development of new antimicrobial agents.

MATERIALS AND METHODS

Plant

The plant specimen with the root of *M. calabura* was identified and certified by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. Voucher specimens were maintained for future reference.

Microorganisms

The selected fungal pathogens including *Alternaria solani*, *Fusarium oxysporum* f.sp *lycopersici*, *Pythium* species, *Phytophthora* species, *Rhizoctonia solani*, *Aspergillus niger* and *Colletotrichum* species used in the present study was obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Extract preparation

The dried and powdered root samples of *M. calabura* L. were extracted by overnight percolation with methanol (polar solvent), chloroform (medium polar solvent) and petroleum ether (least polar solvent) at the rate of 1:5 at room temperature. The extracts were then filtered and concentrated under vacuum in a rotary evaporator to obtain a gummy residue.

Antifungal studies by agar well diffusion assay

The antifungal activity of prepared extracts was tested against selected pathogens on potato dextrose agar. The fungal broth culture 200 µl was transferred to the Petri plates. Using sterile cork borer, wells of 6 mm in diameter were made in the plate containing the media. For each organism, 20 µl of the prepared sample dissolved in ethanol was loaded in each well using sterilized dropping pipette. Three replications were maintained for each treatment. For each microorganism, the positive control (ketoconazole) and the negative control (ethanol) were also loaded in a separate well. The plates were incubated and observed for 2 to 3 days. The diameter of inhibition zone (DIZ) was measured and the mean D1Z was calculated (Ameer et al., 2007; Iqbal and Faiz, 1998).

Fractionation and antifungal assay

The fractionation was carried out by column chromatography using a 22 cm long column having 1.6 cm internal diameter packed with 20 g of silica gel (60 to 120 mesh) using petroleum ether solvent. The active methanol extract was loaded to the packed column and elute with a mixture of petroleum ether and ethyl acetate mixture (90:10) at a rate of 2.6 ml min^{-1}. The polarity of mobile phase was gradually increased using ethyl acetate to get different fractions. Fractions with similar thin layer chromatogram were pooled together which were labeled serially and then subjected to antifungal assay to obtain the active fraction.

Minimum inhibitory concentration (MIC) by tube dilution method

Tube dilution method was used to obtain the MIC of methanol extract of *M. calabura* against selected fungal pathogen (Claeys et al., 1988). The MIC is defined as the lowest concentration of antibiotics or plant extracts that did not show any growth of tested pathogens. The entire test sample were dissolved in ethanol to dilute the highest concentration (10 mg/ml) to be tested and then serial dilutions were made to get 4 different concentrations of 10, 1, 0.1 and 0.01 mg/ml in sterile test tubes containing standardized inoculums. The growth of the organism for each dilution was observed and thus the MIC was evaluated. Ketoconazole and ethanol were used as positive and negative control, respectively.

Spectroscopic studies

The active fraction obtain from the methanol extract was subjected to gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) and infrared spectrometry (IR) spectroscopic studies to identify the structure of the active compound. GC-MS analysis was carried out by using Perkin Elmer - Clarus 500 GC-MS unit. The column used was TR 5-MS Capillary Standard Non-Polar Column with a dimension of 30 Mts, ID of 0.25 mm, film of 0.25 µm and helium as a carrier gas. The flow maintained was 1.0 ml min^{-1} with oven temperature 80°C raised to 280°C at the rate of 10°C min^{-1}. The volume of sample injected was 2 µl. ^{1}H-NMR and ^{13}C-NMR spectrums were recorded at 400 and 100 MHz, respectively in $CDCl_3$. The NMR spectroscopic studies were done by Bruker 400 spectrometer. The Fourier transform infrared (FT-IR) measurement of active fraction was performed using the Nicolet Avatar Model FT-IR spectrophotometer in a diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets.

Molecular modeling studies

Induced Fit Docking (IFD) studies have been carried out using GLIDE (Jitendra and Vinay, 2011) software v5.5, developed by Schrodinger, running on Red Hat Enterprise Linux 5 (RHEL5) workstation and Maestro v9.0 Graphical User Interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation and IFD.

Preparation of the ligand “fungal elicitor cryptogein”

The ligand used in this study was prepared using Ligprep module of v2.3 of Schrodinger Suite 2009. Ligprep follows OPLS-AA (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization. The protein taken for the study was 1LRI (Engineered beta cryptogein complexed with cholesterol) retrieved from PDB database. The optimized structure was then energy

Table 1. Antimicrobial activity of *Muntingia calabura* root extract against fungal plant pathogens.

Extract	Zone of inhibition (Diameter in cm)						
	Alternaria solani	***F.oxysporum* f.sp. *lycopersici***	***Pythium* sp.**	***Phytophthora* sp.**	***Rhizoctonia solani***	***Aspergillus niger***	***Colletotrichum* sp.**
Methanol extract (100 mg/ml)	2.3 (± 0.17)	2.0 (± 0.46)	2.0 (± 0.41)	1.8 (± 0.12)	1.5 (± 0.17)	1.6 (± 0.09)	1.7 (± 0.06)
Chloroform extract (100 mg/ml)	1.5 (± 0.12)	1.4 (± 0.06)	1.5 (± 0.07)	1.2 (± 0.12)	1.0 (± 0.06)	1.2 (± 0.17)	1.2 (± 0.23)
Petroleum ether extract (100 mg/ml)	1.0 (± 0.12)	0.7 (± 0.06)	0.8 (± 0.12)	0.6 (± 0.17)	0.6 (± 0.18)	0.7 (± 0.06)	0.6 (± 0.12)
Ketoconazole (1 mg/ml)	3.5 (± 0.64)	3.0 (± 0.29)	2.8 (± 0.55)	3.0 (± 0.29)	3.2 (± 0.64)	3.5 (± 0.52)	2.9 (± 0.58)
Ethanol (Control)	0.3 (± 0.12)	0.3 (± 0.07)	0.4 (± 0.03)	0.3 (± 0.06)	0.4 (± 0.09)	0.3 (± 0.12)	0.4 (± 0.02)

Values are Mean ± SD of three replications

minimized to remove the steric clashes between the atoms. The energy minimization was done till it reached a Root Mean Square Deviation (RMSD) cutoff of 0.18 Å and the resulting structure was used for docking (Thangaraj, 2011).

Induced fit docking (IFD)

IFD of the prepared ligand with the prepared protein was performed using IFD protocol of GLIDE v5.5 from Schrodinger Suite 2009. Both the ligand and the receptor were flexible which enabled the ligand to dock at the receptor's binding site and generate multiple poses of the receptor-ligand complex. Each docking included unique structural conformations of the receptor needed to fit the ligand pose. The IFD gives the best structure of the docked complex based on the Glide score (G-score) of the dockings.

RESULTS

The root of *M. calabura* L. possesses several secondary metabolites with remarkable biological properties. With this background the present research has been initiated to identify the active secondary metabolite compound produced by *M. calabura* L. root against selected plant pathogens.

Antifungal activity and fractionation

The various extracts of *M. calabura* root were screened for their antifungal activity against selected plant pathogens. Among the three extracts tested, methanol extract exhibited the highest zone of inhibition when compared with chloroform and petroleum ether extract at a concentration of 100 mg/ml against the selected pathogens. The diameter of inhibition zone produced by the methanol extract (Table 1) was comparable to that of the positive control ketoconazole. The crude extract obtained from methanol extract of *M. calabura* roots were subjected to column chromatography for purification purpose. The polarity of petroleum ether was gradually increased using ethyl acetate up to 82:18 (Petroleum ether:Ethyl acetate) to give 40 different fractions. Fractions with similar TLC chromatogram were pooled together and as a result a total of 23 different fractions were obtained which were labeled as F1-F23. The collected fractions were tested for their antifungal activity against selected plant pathogens and the findings revealed that fraction No. 21 exhibited maximum antifungal activity when compared with other fractions (Table 2).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration assay was carried out for isolated antifungal fraction F21 against selected plant pathogens. The bioactive fraction F21showed inhibition at a concentration of 10 and 1 mg/ml against the pathogens *A. solani*, *F. oxysporum* and *Phytophthora* spp., while growth was observed in the other two dilutions 0.1 and 0.01 mg/ml (Table 3).

Spectroscopic studies

The antifungal compound isolated from the root of *M. calabura* L. was obtained as whitish slight yellow colored compound. The spectroscopic studies confirmed the isolated active metabolite as stigmasterol. The molecular formula was determined as $C_{29}H_{48}O$ by GC-MS studies (Figure1) with m/z value of 412. The fragmentation pattern confirmed the presence of stigmasterol. The IR absorption spectrum showed absorption peaks at 3470 cm^{-1} (O-H stretching), 2867.9 cm^{-1} (aliphatic C-H stretching), and 1704.6 cm^{-1} (C=C absorption peak). These absorption frequencies resemble the absorption frequencies observed for stigmasterol (Figure 2). In the ^{1}H-NMR spectrum, the six methyl groups appeared as, two singlet of three proton integration at δ 0.68 and 0.98 and were assigned to H-18 and H-19 of the tertiary methyl groups.

Table 2. Antifungal activity of the fraction separated from ethanolic extract of *M. calabura* root extract by column chromatography against fungal pathogens by agar well diffusion assay.

Fraction No.	Diameter of inhibition zone in cm					
	A. solani	***F. oxysporum* f.sp. *lycopersici***	***Pythium* sp.**	***Phytophthora* sp.**	***A. niger***	***R. solani***
F1	-	-	-	-	-	-
F2	-	-	-	-	-	-
F3	-	-	-	-	-	-
F4	-	-	-	-	-	-
F5	-	-	-	-	-	-
F6	-	-	-	-	-	-
F7	-	-	-	-	-	-
F8	-	-	-	-	-	-
F9	-	-	-	-	-	-
F10	-	-	-	-	-	-
F11	-	-	-	-	-	-
F12	-	-	-	-	-	-
F13	-	-	-	-	-	-
F14	0.2	0.2	0.2	0.2	0.2	0.2
F15	0.3	0.2	0.3	0.2	0.2	0.2
F16	-	-	-	-	-	-
F17	-	-	-	-	-	-
F18	-	-	-	-	-	-
F19	-	-	-	-	-	-
F20	0.4	0.3	0.3	0.3	0.3	0.3
F21	3.2	3.0	2.5	2.1	1.9	1.7
F22	0.3	0.2	0.3	0.3	0.3	0.3
F23	-	-	-	-	-	-
Ethanol (Control)	0.3	0.4	0.3	0.4	0.3	0.3
Ketoconazole (1 mg/ml)	3.5	3.1	2.8	3.1	2.4	3.2

Three secondary methyl groups resonated as doublets at δ 1.00 (J=6.5 Hz), δ 0.82 (J=6.0 Hz) and δ 0.77 (J=6.0Hz), and were assigned to H-21, H-26, H-27, respectively, whereas one primary methyl group appeared as triplet at δ 0.78 (J=7.5 Hz) and was ascribed to the H-29 of the side chain. The appearance of a one proton multiplet at a downfield value of δ 3.51 in the ^{1}H-NMR spectrum revealed the presence of tertiary proton attached to hydroxyl group (Figure 3). Moreover, the ^{13}C-NMR indicated 29 carbon signals. The corresponding carbon signal, that is, C-3 was indicated at δ 71.8 in the ^{13}C-NMR spectrum. The presence of a steroid skeleton was further confirmed by the ^{13}C-NMR signals at δ 130.2 and 138.3 and δ 140.7 and 121.7. ^{13}CNMR (Figure 4) ($CDCl_3$, 100MHz): ^{13}C-NMR has given signal at 139.8 (C-5), 137.6 (C-22), 118.3, (C-6), 71.1 (C-3),

Table 3. Minimum inhibitory concentration of stigmasterol isolated from *M. calabura* root extract against fungal plant pathogens.

Extract	Fungal plant pathogens						
	Alternaria solani	***F.oxysporum* f.sp. *lycopersici***	***Pythium* sp.**	***Phytophthora* sp.**	***Rhizoctonia solani***	***Aspergillus niger***	***Colletotrichum* sp**
Stigmasterol (mg/ml)							
10	-	-	-	-	+	+	+
1	-	-	+	-	+	+	+
0.1	+	+	+	+	+	+	+
0.01	+	+	+	+	+	+	+
Ketoconazole (mg/ml)							
10	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-
0.1	+	+	+	+	+	+	+
0.01	+	+	+	+	+	+	+
Solvent control	+	+	+	+	+	+	+
Cells	+	+	+	+	+	+	+

+ Growth; - No growth.

55.3(C-14), 55.18(C-17), 50.45 (C-9), 48.3 (C-9), 40.8 (C-20), 40.1(C-12),39.2 (C-13), 38.9 (C-4), 38.6 (C-12), 37.18 (C-1), 37.12 (C-10), 36.3(C-8), 35.59(C-20), 34.29 (C-22), 34.24 (C-7), 32.66 (C-8), 29.86 (C-25), 29.71 (C-16), 28.2 (C-2), 28.1 (C-15), 27.4 (C-28), 26.1 (C-11,26), 21.6 (C-27), 19.32 (C-19), 17.71 (C-21), 15.6 (C-18, 29), 130.2 (C-23). All the spectral data and previous literature reveal that the compound is stigmasterol (Figure 5).

Docking studies

IFD between the target protein beta-cryptogein (PDB ID-1LRI) and the compound stigmasterol was carried out using glide and the images are obtained using Pymol. This study clearly indicates that stigmasterol can be easily bind with the cryptogein elicitors secreted by the pathogens and can prevent plant from pathogen attack. The possible conformations of compound stigmasterol along with their docking score and glide energy are shown in Table 4. The stigmasterol binds with cryptogein elicitors with a docking score value of -12.25 and glide energy of -42.56. The interaction was done in chain A Tyr 47 [O – H…O] residue with a distance of 2.7 A° (Figure 6).

DISCUSSION

The root of *M. calabura* L. is a commonly used botanical medicine. The present study has explored the use of *M. calabura* in the field of agriculture, by using it against selected plant pathogens. A new antifungal compound, stigmasterol was identified with a minimum inhibitory concentration of 1 mg/ml against the selected pathogens, namely, *A. solani*, *F. oxysporum* f.sp. *lycopersici,* and *Phytophthora* spp.

There is growing evidence that most of the secondary metabolites from plants are involved in the defense of the plant from plant pests and diseases. Thus, secondary compounds represent a large reservoir of chemical structures with biological activity. This resource is largely untapped for use as pesticides and fungicides. Many of the medicinal plants have been found to possess antimicrobial activity against an array of plant pathogens (Okigbo and Nmeka, 2005; Siva et al., 2008). Some plants may be alternatives to currently used disease control agents, since they constitute a rich source of bioactive chemicals (Swain, 1977). The substances, which can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered as candidates

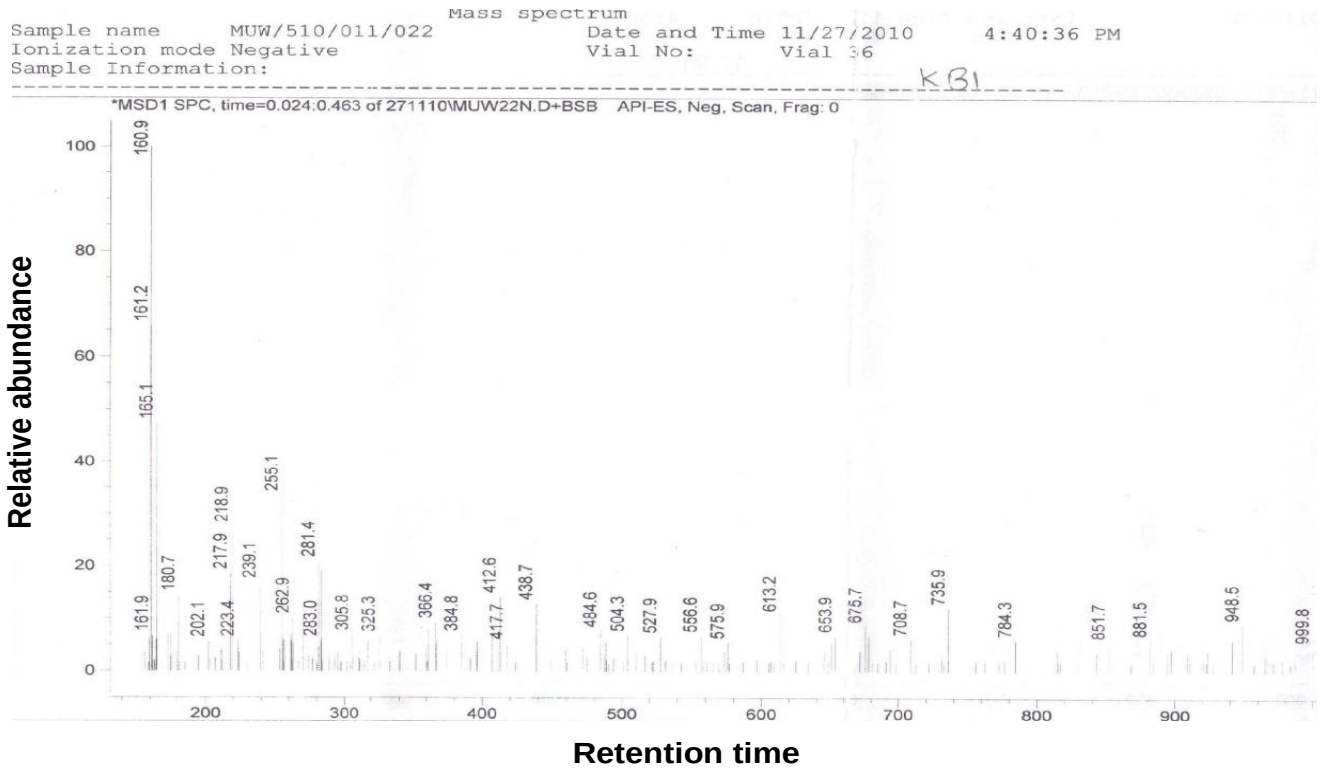


Figure 1. Gas chromatographic mass spectrum of purified antimicrobial fraction F21 from *M. calabura* root extract.

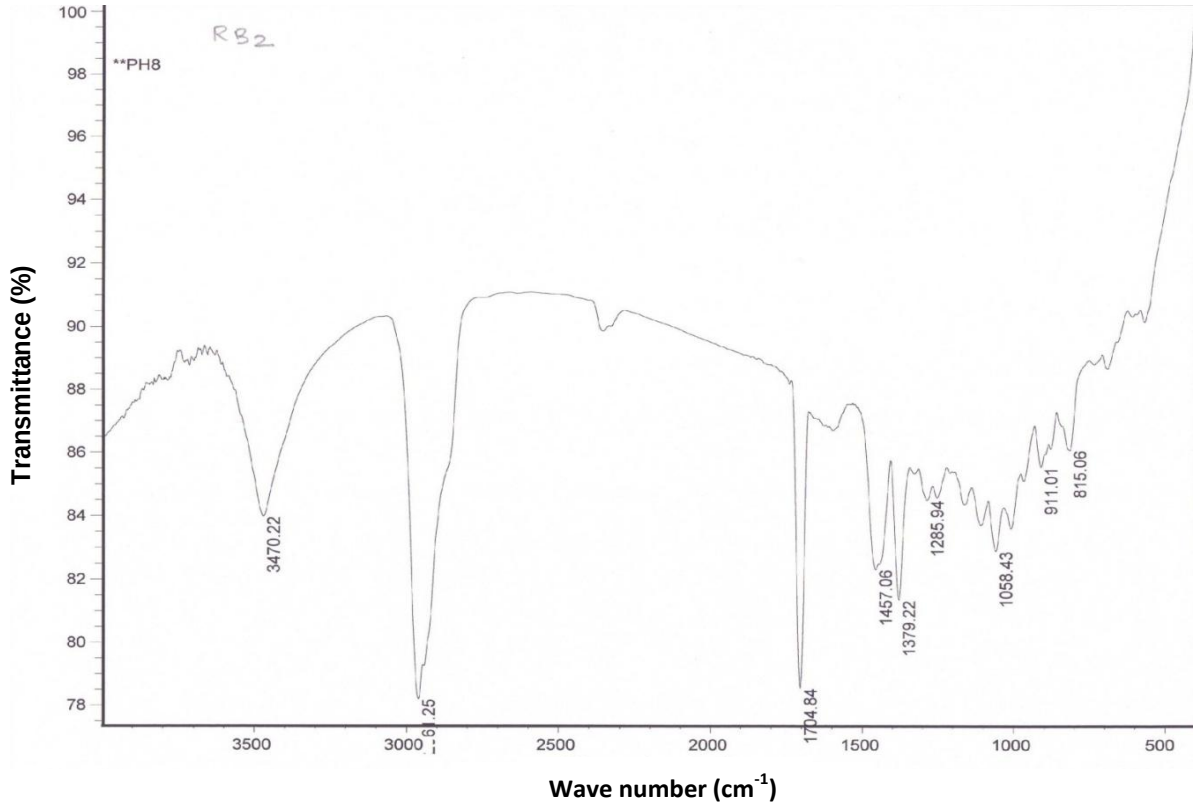


Figure 2. Infrared (IR) spectrum of purified antimicrobial compound from *M. calabura* root extract.

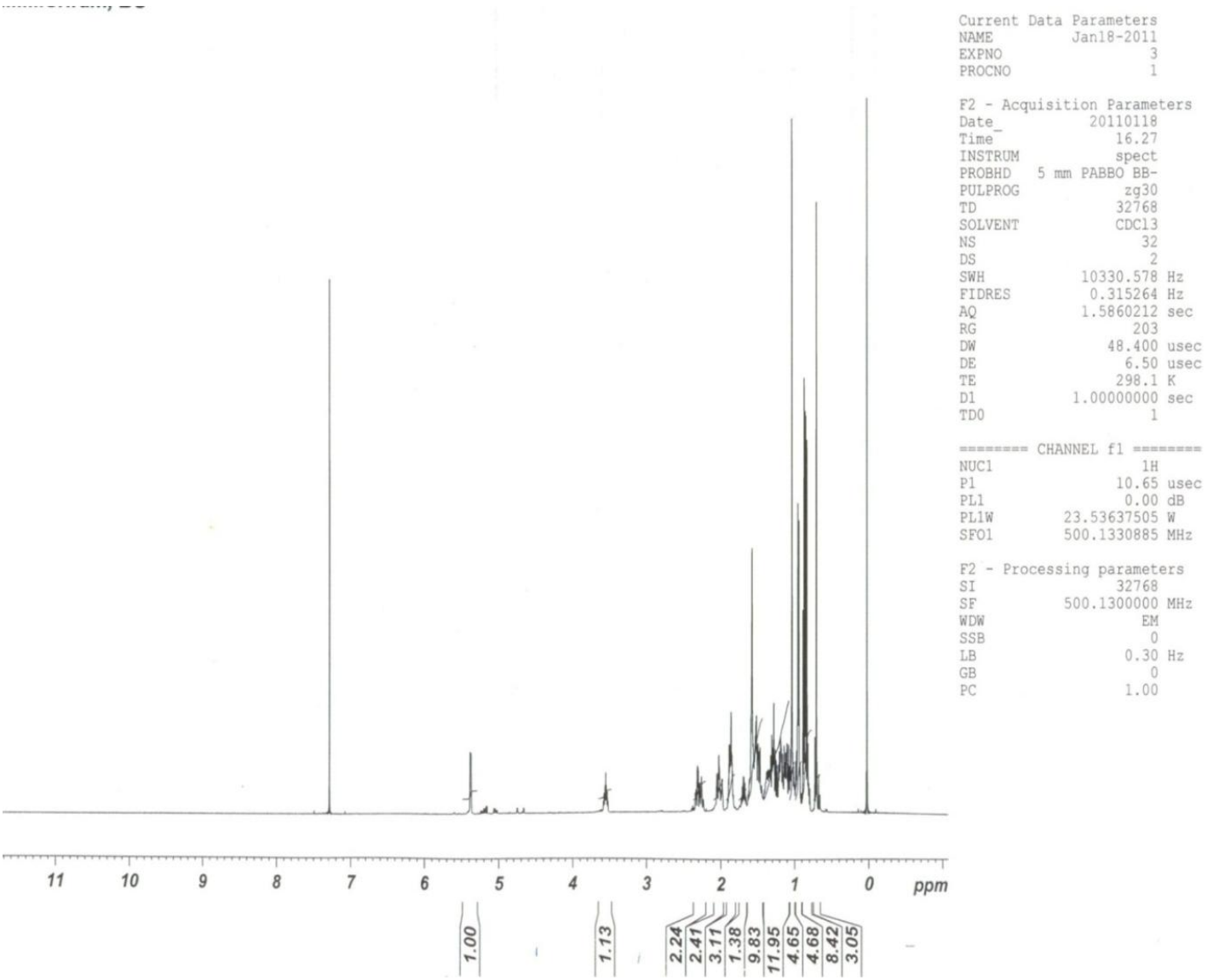


Figure 3. ^{1}H NMR studies of purified antimicrobial fraction F21 from *M. calabura* root extract.

Determination of antimicrobial activity of *M. calabura* root was tested against selected fungal plant pathogens by agar well diffusion assay. Methanol extract of *M. calabura* exhibited considerable antimicrobial activity against fungal plant pathogens compared to other solvent extracts.

Further, the methanol extract of *M. calabura* root were subjected to column chromatography for the purpose of isolating purified antimicrobial compounds. The methanol crude extract were separated into 23 fractions in column chromatography. The fraction 21 obtained exhibited higher antimicrobial activities against the tested plant pathogens as compared to other fractions. The maximum inhibitory activity in drop diffusion assay is observed for *A. solani* and *F. oxysporum* f.sp*. lycopersici* with a DIZ of 3.2 and 3.0 cm observed against, respectively.

Asiaticoside (the ester glycoside of the triterpene asiatic asiatic acid) and madecassoside (the ester glycoside of the triterpene madecassic acid) are the chief medicinally active compounds of the plant *Centella asiatica* (“Mandukparni”) and *Datura stramonium*. These were successfully extracted through column chromatography along with a saponin fraction. The compounds were isolated from other plant constituents by methanol extraction and separated from other terpenes by silica gel column chromatography (Matsuda et al., 2001).

Gas chromatography mass spectrometry identifies the compounds based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns. In our present investigation, gas chromatography mass spectrometry studies were carried out for purified antimicrobial fraction obtained from column chromatography

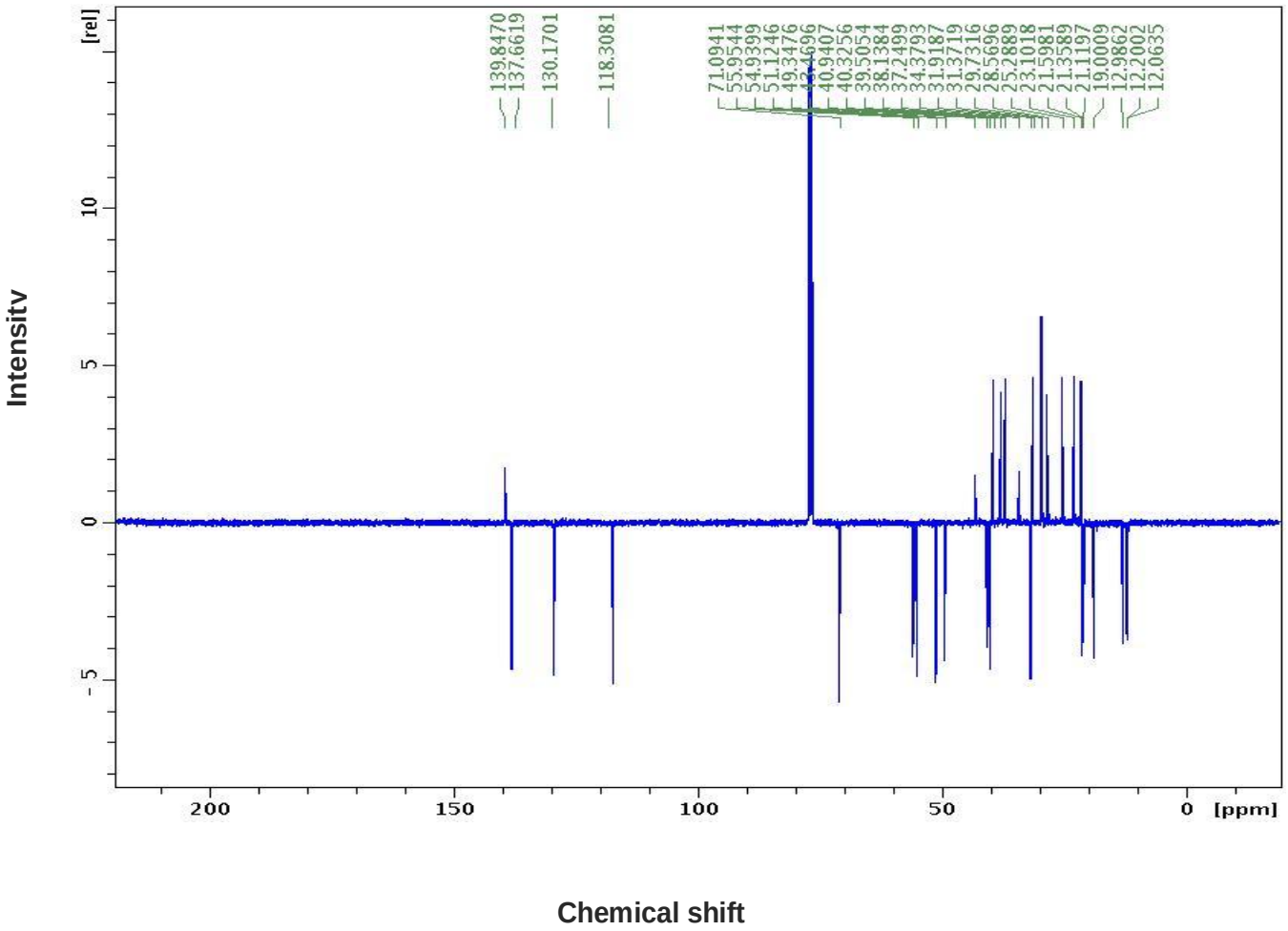


Figure 4. ^{13}CNMR studies of purified antimicrobial fraction F21 from *M. calabura* root extract.

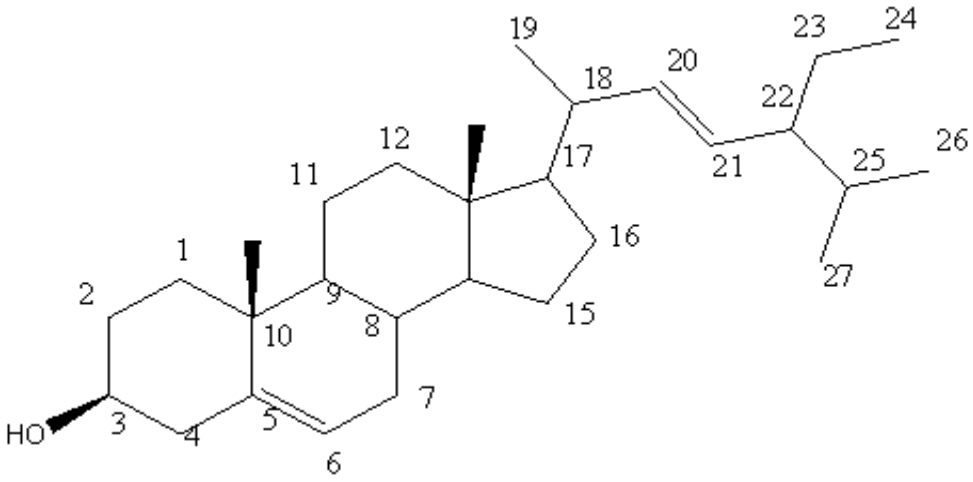
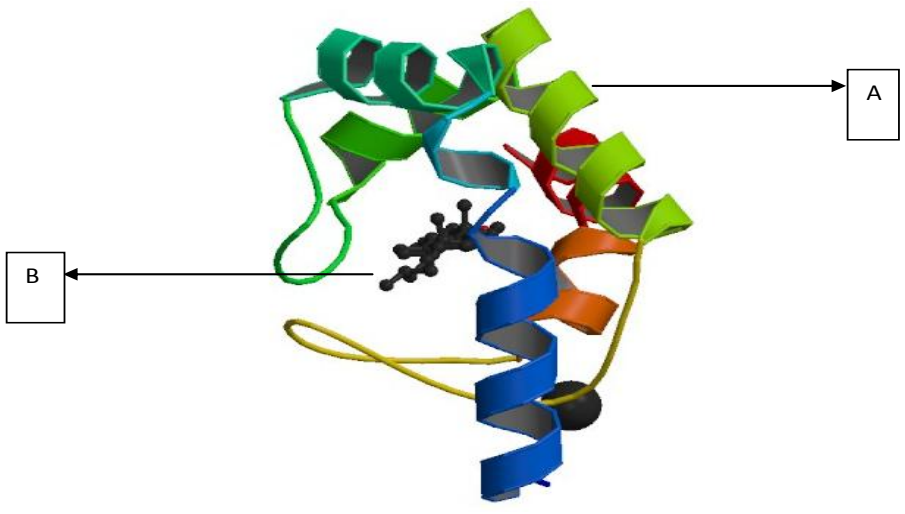


Figure 5. Structure of stigmasterol obtained from *M. calabura* root extract. Molecular weight, 412.70; Formula, $C_{29}H_{48}O$; IUPAC name, (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(*E*,2*R*,5*S*)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol.

Table 4. Induced fit docking results of the compound stigmasterol against the target fungal elicitor Beta-Cryptogein.

Pose	Docking score	Glide energy	Interaction	Distance (Å)
1	-12.59	-42.65	TYR47(O-H...O)	2.70
2	-11.85	-46.75	(O-H...O)TYR87 TYR47(O-H...O)	3.04 3.00
3	-12.04	-44.16	(O-H...O)TYR87 TYR47(O-H...O)	2.94 2.76
4	-12.04	-43.64	TYR47(O-H...O)	2.80
5	-11.43	-44.27	(O-H...O)TYR87	2.91
6	-11.98	-44.55	(O-H...O)TYR87	3.00
7	-11.34	-46.48	(O-H...O)TYR87 TYR47(O-H...O)	2.90 2.88
8	-11.29	-41.80	LYS94(N-H...O)	2.96
9	-11.64	-44.37	(O-H...O)TYR87 TYR47(O-H...O)	3.24 2.99
10	-11.31	-44.89	(O-H...O)TYR87 TYR47(O-H...O)	2.99 2.92
11	-10.78	-43.81	(O-H...O)TYR87	3.27
12	-10.66	-41.73	(O-H...O)TYR87 TYR47(O-H...O) LYS94(N-H...O)	3.09 3.06 3.13
13	-10.63	-45.48	TYR47(O-H...O)	2.72
14	-10.41	-42.01	(O-H...O)TYR87 TYR47(O-H...O)	3.18 2.93



a - Stigmasterol
b - Fungal elicitor and stigmasterol interaction site.(Try 47 (O-H…O) residue

Figure 6. Molecular docking of stigmasterol (*M. calabura* root) compound with fungal elicitor crptogein in GLIDE software.

(fraction 21). The mass spectrum of the fraction 21 showed molecular ion peak at m/z 412 associated with the molecular formula $C_{29}H_{48}O$ and consistent with six degrees of unsaturation. The library match of the GC-MS showed that the compound stigmasterol was present in the methanol extract of *M. calabura* root.

^{1}H NMR and ^{13}C NMR spectroscopic studies were carried out for the purified antimicrobial fraction 21 from methanol root extract of *M. calabura*. The spectrum depicts that the isolated fraction is stigmasterol based on their chromatogram pattern. The spectrum of the stigmasterol is confirmed with previous literature collection (Anjoo and Ajay, 2011). The assignment of carbon signals in ^{13}C NMR and the location of substituent in proton NMR and GC-MS suggested that the compound was found to be stigmasterol.

In the present study the IR absorption spectrum showed absorption peaks at 3470cm^{-1} (O-H stretching.); 2867.9cm^{-1} (aliphatic C-H stretching); 1704.6cm^{-1} (C=C absorption peak). These absorption frequencies resemble the absorption frequencies observed for stigmasterol.

Based on the IR spectroscopic studies, the stigmasterol produced absorptions at 3373.6 cm^{-1} characteristic of O-H stretching, 2940.7 and 2867.9 cm^{-1} is due to aliphatic C-H stretching. Other absorption frequencies include 1641.6 cm^{-1} as a result of C=C stretching, however this band is weak; 1457.3 cm^{-1} is a bending frequency for cyclic $(CH_2)_n$ and 1381.6 cm^{-1} for $–CH_2(CH_3)_2$. The absorption frequency at 1038 cm^{-1} signifies cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 881 cm^{-1}. Similar kinds of absorption frequencies are observed for fraction 21 which confirms the isolated antimicrobial metabolite as stigmasterol.

The stigmasterol is found as a free or compound in the cell. They are structural components of the lipid core of cell membrane and being precursor of numerous secondary metabolites including plant steroid hormones (Noguchi et al., 2000)

Stigmasterols possess several biological activities, but they also play an important role in defense mechanisms against insects and plant pathogens. An increasing body of evidence indicates that the chemical interactions between plant-pathogenic fungi and higher plants are both complex and highly integrated. For instance, as fungi have developed toxins that increase their virulence on plant tissues, plants have developed a variety of ways to limit the effectiveness of these fungal toxins. Biosynthesis of fungal toxins can, however be blocked *in vitro* by the addition of certain naturally occurring plant metabolites at concentrations inhibitory to fungal growth.

Protein-Ligand Docking in drug discovery and development, the manner in which small-molecule compounds bind or dock with proteins is of utmost importance. Proteins are often the main targets for new drugs; many drug compounds are small molecules that are designed to bind preferentially to specific proteins. Because of this, small molecules need to be design for protein docking; many bioinformatics tools exist for the analysis of protein-ligand interactions. These tools often fall in the category of computational chemistry. Databases and bioinformatics tools containing genomic, proteomic and functional information have become indispensable in antimicrobial drug research. Many general databases are used in this field, such as Uniport and Protein Data Bank (PDB) (Hughes et al., 1990).

The stigmasterol compound isolated from methanol root extract of *M. calabura* was subjected to protein docking studies with fungal elicitor cryptogein. In the fungal elicitor cryptogein, interaction was observed for TYR-47 with hydrogen bonding interactions only during the induced fit docking in most of the poses. Hence, the docking studies suggest the anti-fungal nature of stigmasterol. The minimum value of the docking score and glide energy obtained for stigmasterol show the binding stability of the compound.

Docking has been used to discover novel ligands for well over 30 targets. The inhibitors discovered were novel, having little similarity to the known ligands. Most initial leads had affinities in the low-micromolar range. The new sulphonamide inhibitors of carbonic anhydrase are the exception; they are much more potent. There has been an efflorescence of new docking methods in the past several years and several docking programs were used in the studies (Shoichet et al., 2002).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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