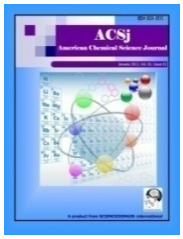

American Chemical Science Journal
4(3): 286-297, 2014

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Isolation and Characterization of a Bioactive Phenylpropanoid from *Ocimum sanctum* L. Leaves through Chromatographic and Spectroscopic Methods

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Authors' contributions

This whole work was carried out in collaboration between all authors. Finally all authors read and approved the final manuscript.

Original Research Article

Received 28th August 2013
Accepted 1st October 2013
Published 15th January 2014

ABSTRACT

Aims: This study aims at characterization of purified active ingredient isolated from commonly available botanical plant, *Ocimum sanctum* L. and to assess its potential for deployment in integrated disease management strategy of a staple food crop rice (*Oryzae sativa* L.) and rice based crop groundnut (*Arachis hypogea*) as an environment friendly approach for management of serious diseases of these two crops in lieu of synthetic chemicals.
Study Design: Spectrophotometric and chromatographic methods were used. Bioassay experiments were designed as per Complete Randomized Design (CRD) and statistical analysis was done using Cropstat 7.2 developed by International Rice Research Institute (IRRI).
Place and Duration of Study: Laboratory of Natural Plant Products, Crop Protection Division, Central Rice Research Institute, Cuttack-753006 (Indian Council of Agricultural Research), Odisha, India.
Methodology: Partial purification of active principle was conducted through Column Chromatography in polarity gradient solvent systems followed by single spot isolation of active compound and mass collection through Thin Layer Chromatography (TLC).For characterization, Spectroscopic techniques viz., UV-Vis, FT-IR, ^{1}H-NMR, ^{13}C-NMR, DEPT,

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Mass and elemental analysis were conducted. For screening of different partially purified fractions of column against *Pyricularia grisea*, Conidial Germination Test method was preferred. To assess the fungitoxic activity of active principle against mycelial growth of five phytopathogenic fungi viz., *P. grisea*, *Helminthosporium oryzae*, *Rhizoctonia solani*, *Curvularia lunata* and *Aspergillus niger*, Poisoned Food Technique was used.

Results: FT-IR data of active compound was in conformity with FT-IR of eugenol available in literature, ^{1}H-NMR revealed presence of 12 protons, ^{13}C-NMR showed the presence of 10 C atoms, DEPT along with ^{13}C-NMR corresponds to the presence of substitution at 1, 2 and 4 positions of benzene ring. Mass spectrum reveals the presence of base peak at 164 that corresponds with the molecular weight of eugenol. Elemental analysis showed that percentages of C, H and O in the isolated compound were in conformity with calculated values of those in eugenol. The compound was found to exhibit broad spectrum fungitoxicity against five fungal pathogens tested.

Conclusion: The isolated and purified compound from *O. sanctum* (Krishna i.e. Black) was characterized as Eugenol. It may be further used as an eco-friendly organic fungicide but only after developing suitable formulation and its evaluation under *in-vivo* conditions.

Keywords: Ocimum sanctum; spectroscopy; chromatography; eco-friendly; eugenol, fungitoxic; phytopathogenic fungi.

1. INTRODUCTION

Ocimum sanctum L. (Krishna i.e.) belonging to family Lamiaceae, possess various medicinal properties ranging from antimicrobial, antidiabetic, hepato-protective, anti-inflammatory, anticarcinogenic, neuroprotective, cardioprotective, mosquito repellent etc. [1]. Earlier phytochemical work has resulted in the isolation of monoterpenes, oxygenated monoterpenes, sesquiterpenes and phenylpropenes [2]. Besides, possessing antibacterial, insecticidal and nematicidal properties [3, 4, 5], in previous studies *O. sanctum* extracts have been found to be fungitoxic against phytopathogenic fungi *Pyricularia grisea* Sacc., *Rhizoctonia solani* Kühn and *Aspergillus niger* Van Tieghem [6, 7] . It was thought pertinent to isolate the bioactive principle from the essential oil and test its spectrum of fungitoxic properties against certain important fungal pathogens of rice viz. *P. grisea*, *Helminthosporium oryzae* Breda de Haan, *R. solani*, *Curvularia lunata* (Wakker) Boedijin inciting destructive diseases viz., Blast, Brown-spot , Sheath blight and Grain discoloration in storage respectively and on a rice based crop groundnut viz. *A.niger* causing Collar-rot disease, with the aim to develop an eco-friendly alternative to the synthetic chemicals to avoid undesirable impact on environment and potential health risks [8].

2. METHODOLOGY

2.1 Plant material and Extraction of Essential Oil

Fresh leaves of *O. sanctum* weighing 1 Kg were hydro-distilled (1:1w/v) through Clevenger's apparatus. Essential oil (5 ml) was collected and moisture from the oil was separated utilizing differential freezing point principle as per method given by Tewari et al. [7]. The remaining moisture was removed by addition of Sodium Sulphate (Na_2SO_4) and pure essential oil was decanted in a clean sterilized glass vial.

2.2 General Experimental Procedures

Column chromatography was performed using glass column (113 cm x 5.7 cm) packed with hexane saturated silica gel (60-120 mesh size, Merck) up to 81 cm ($2/3^{rd}$) height from the bottom.

For repeated purification and mass collection of the active principle, Thin Layer Chromatography (TLC) was done using Silica gel G (Batch no.QD2Q621185 and Product No.6173105001730 from Merck). Clean glass chromatographic plates (8x8 cm^2) were utilized as supporting base over which the slurry (silica gel G and double distilled water in the ratio w/v) was uniformly coated by spreading with the help of applicator to a 2 mm thickness.

FT-IR was recorded on Perkin Elmer Spectrum RX1. ^{1}H-NMR ($CDCl_3$, 400.1MHz), UV spectrum was recorded by Cecil CE 7400 Spectrophotometer. ^{13}C-NMR ($CDCl_3$, 100.6MHz) and DEPT spectra were recorded by FT-NMR Spectrometer, BrukerAvance II. Chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. Mass spectrum of the compound was recorded by Mass Spectrometer, Waters Micromass Q-Tof Micro. Elemental analysis was carried out on Elementar Vario EL III.

2.3 Isolation of Fungal Pathogens

Pure culture of *P. grisea*, *H. oryzae*, *R. solani*, *C. lunata* and *A. niger* were isolated from susceptible rice cultivar viz. HR-12, Benibhog, Karuna and Utkalprava and groundnut cultivar AK12-24 respectively. The pathogenicity of the all five phytopathogenic fungi was confirmed through Koch's postulate.

2.4 Isolation Process and Purification of Bioactive Compound

Bioactive principle from *O. sanctum* essential oil was partially purified through Column Chromatography in polarity gradient based solvent systems viz. hexane, hexane+ benzene (50:50), benzene, benzene+ chloroform (50:50), chloroform, chloroform + methanol (50:50) + methanol, methanol + acetone (50:50), acetone alone in a dust proof chamber at 25 ±2ºC. Each of the chromatographed elutes @ 25 ml collected separately in clean conical flask were bio-assayed against the key pathogen, *P. grisea* through Conidial Germination Test as per method described by Nene and Thapliyal [9] to monitor and locate movement of active principle in these. Hexane fractions were selected for further isolation and purification of active compound as larger number of elutes in this solvent were found to exhibit highest fungitoxic activity i.e. complete inhibition of conidial germination.

Amongst different solvent systems tried to make better chromatographic separation of the mixture of compound present in hexane elute of the column fraction, Benzene was utilized as developer solvent owing to better separation of active principle than any other tried.

Hexane elutes exhibiting similar and highest fungitoxic activities were pooled together and further proceeded for single spot isolation of active compound through Thin Layer Chromatography. Concentrated solution of active hexane fractions were spotted on activated silica gel plate that was placed in chamber saturated with a few crystals of iodine. Spots, tan-brown in colour appeared at R_f= 0.66. Further, spots from similarly developed and dried other TLC plates at the same R_f (0.66) were scrapped out for the mass collection of active

compound (Fig. 1). The presence of the active compound in the scrapped out silica gel was confirmed through bioassay test. The fractions eluting with 100% hexane yielded pure compound as pale yellow oil (3.01 g).

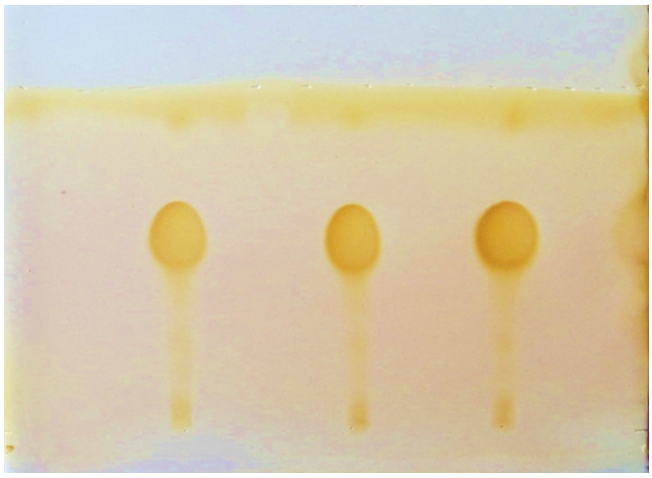

Fig. 1. TLC plate displaying purified active compound spot

2.5 Data interpretation

2.5.1 UV-Vis data

Solution of active compound in acetone exhibited λ_{max} at 268 nm at O.D. 1.1.

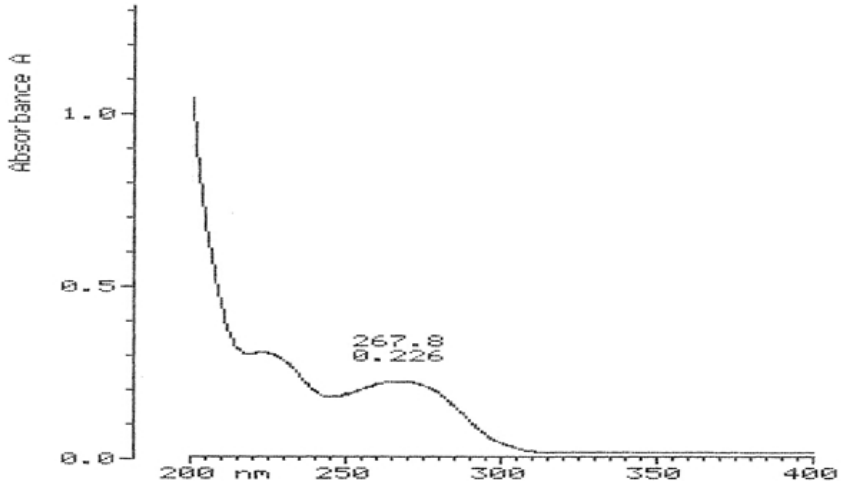

Fig. 2. UV spectrum of compound isolated from *O. sanctum* essential oil

2.5.2. FT-IR data

FT-IR of the isolated active compound showed $\bar{\upsilon}$ (cm^{-1}) : 3514 (Phenolic OH stretching), 3076 (C-H stretching in aromatic ring), 3003 (C-H stretching in =C-H, olefinic), 2975, 2937, 2842 (C-H stretching, aliphatic), 1637 (C=C in allyl group), 1612, 1514, 1464, 1451(sharp, C=C in aromatic ring)1432 (sharp, CH_2, aliphatic), 1366 (sharp, CH_3), 1267, 1234 (C-O-C str., asymmetric), 1034 (C-O-C stretching symmetric), 794, 746 (C-H outplane bonding), 648 (C=C outplane bonding) .

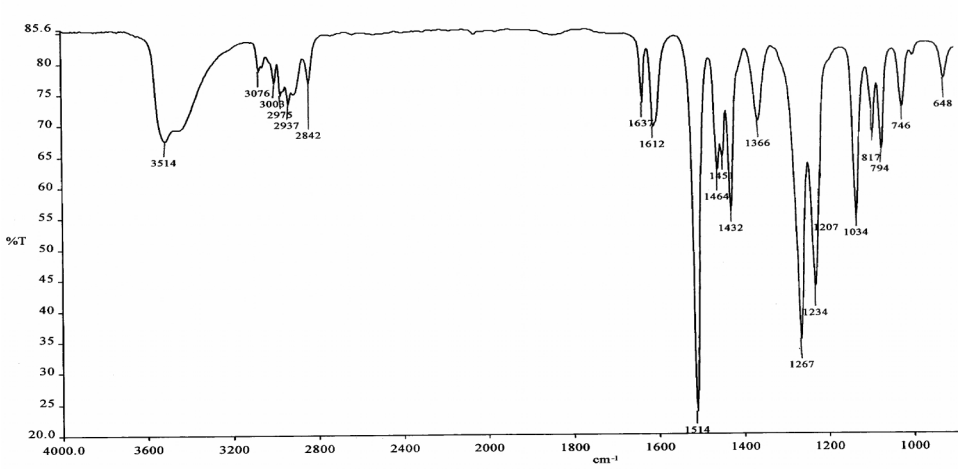

Fig. 3. FT-IR spectrum of compound isolated from O. sanctum essential oil

2.5.3 ^{1}H- NMR data

^{1}H- NMR data showed δ : 3.33-3.31 (d, 2H, J=16.7 Hz, C-7 H), 3.87 (s, 3H, C-10 H), 5.09 - 5.03 (m, 2H, C-9 H), 5.49 (s, 1H, Ph-OH), 6.0-5.89 (m, 1H, J= 8.3 Hz, C-8 H), 6.69 - 6.67 (m, 1+1 H, C-3 H, C-5 H), 6.85-6.83 (d, 1H, J=10.6 Hz, C-6 H).

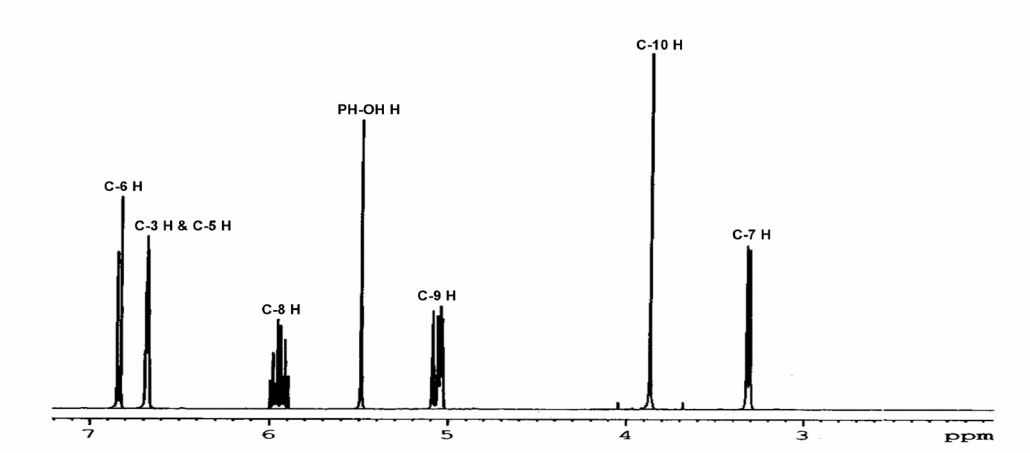

Fig. 4. ^{1}H- NMR spectrum of compound isolated from *O. sanctum* essential oil

2.5.4 ^{13}C-NMR data

^{13}C-NMR showed δ: 39.9 (C-7), 55.87 (C-10), 143.89 (C-1), 146.43 (C-2), 111.09 (C-3), 131.93 (C-4), 121.18 (C-5), 114.24 (C-6), 115.34 (C-9), 137.8 (C-8)

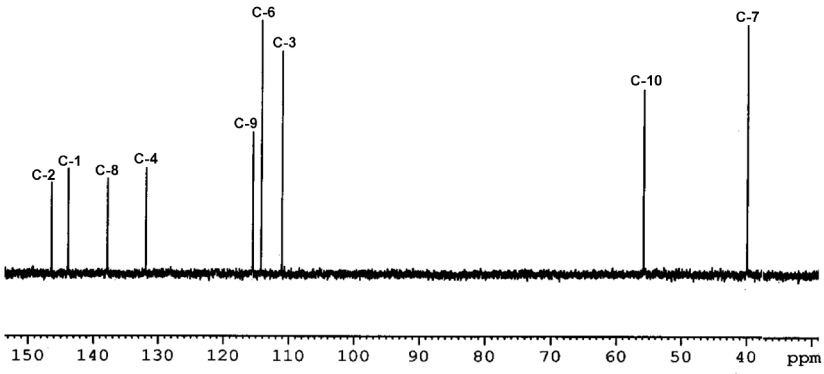

Fig. 5. ^{13}C-NMR spectrum of compound isolated from *O. sanctum* essential oil

2.5.5 DEPT data

DEPT spectrum showed one primary, four tertiary carbons and two secondary carbon.

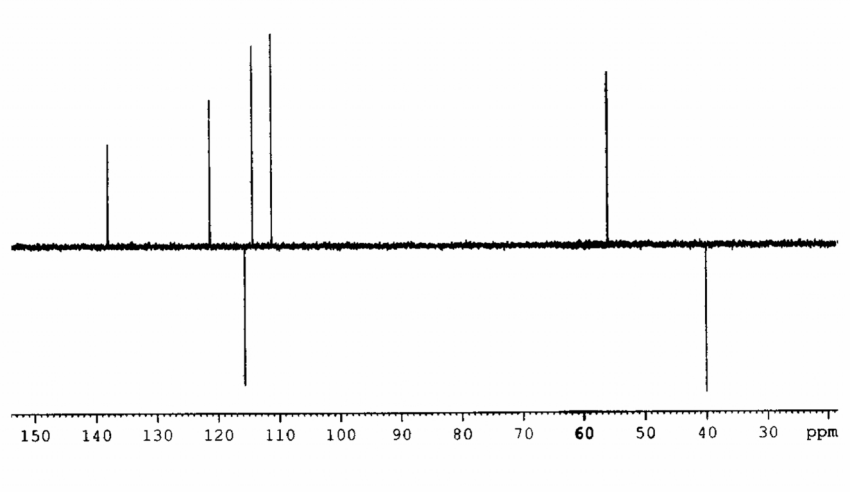

Fig. 6. DEPT-NMR of compound isolated from *O. sanctum* essential oil

2.5.6 Mass analysis data

Mass spectrum showed m/z 164 [$M^{\cdot+}$, 100%], 149 [$(M\text{-}CH_3)^+$], 137 [$(M\text{-}CH{=}CH_2)^+$], 121 [149-CO]$^+$. Other important peaks were observed at m/z 131, 103, 91 and 77.

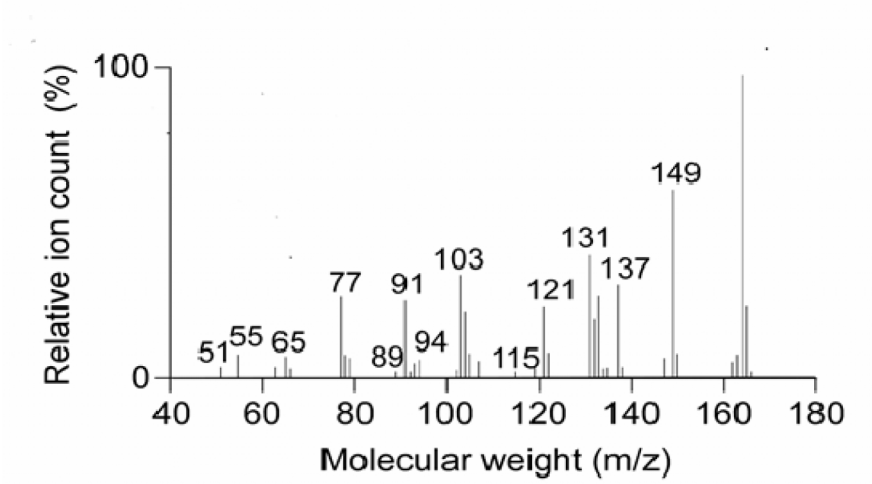

Fig. 7. Mass spectrum of the compound isolated from *O. sanctum* essential oil

2.5.7 Elemental analysis data

Table 1. Percentage of elements in compound isolated from *O. sanctum* essential oil

Element	Percentage
Carbon	73.11 (73.14)*
Hydrogen	7.35 (7.38)*
Oxygen	19.50 (19.48)*

**represents calculated values*

2.6 Fungitoxic Activity of Bioactive Compound Isolated from *O. sanctum* Essential Oil against Phytopathogenic Fungi of Rice and Rice Based Crop Groundnut

The isolated active compound was tested for its fungitoxicity against mycelial growth of phytopathogenic fungi viz. *P. grisea*, *H. oryzae*, *R. solani*, *C. lunata* of rice and *A. niger* of groundnut crops through poisoned food technique as per method described by Nene and Thapliyal [9]. In this technique, active ingredient (10% concentration in ethanol) was combined with molten Oat Meal Agar (in case of *P. grisea*) / Potato Dextrose Agar media (in case of rest of the pathogens) separately so as to get the final concentration of 1%, 0.1%, 0.01 and 0.001% . The extract mixed media was poured into the petri plates aseptically and inoculated after 4 days allowing ethanol to be evaporated from the media meanwhile, as also in control plate. Actively growing mycelia of the test pathogen was cut with a sterile cork-borer and inoculated separately in the center of each such petri plates aseptically. All plates were incubated at 28±2ºC for five to fifteen days depending on the test pathogen inoculated (five days for *R. solani*, fifteen days for *P. grisea* and seven days for *H. oryzae*, *C. lunata* and *A. niger*). Experiment was repeated thrice keeping three replications each time. The mycelial

growth (cm) was observed and recorded when it grew to periphery in control petri plates and was computed through $3.14 \times r^2$ method [10]. Whereas, in case of *A. niger* due to its patches of growth, grown patches were drawn on transparent polythene sheet on cultured petri plates and growth was computed through graph paper. No mycelial growth was accorded numerical value 0.2 cm^2, for the purpose of statistical analysis done throughCropstat 7.2 developed by International Rice Research Institute (IRRI).

3. RESULTS AND DISCUSSION

3.1 Data Interpretation

3.1.1 UV data

UV spectra of the purified active compound isolated from the essential oil of *O. sanctum* (Krishna) exhibited maximum absorbance at 268 nm (OD = 1.1) indicating the presence of allylic group attached to the benzene ring (Fig. 2). Moreover the molecule gave low energy transition compared to alkyl moieties. In literature, UV spectra of eugenol showed λ_{max} at 275 nm at absorbance=1.4 which confirms that the bioactive compound isolated from *O. sanctum* leaves essential oil may be eugenol [11].

3.1.2 FT-IR data

The FTIR of the active compound isolated from *O. sanctum* leaves essential oil showed a peak at 3514 cm^{-1} indicating to phenolic OH stretching (Fig. 3). Presence of unsaturation is confirmed by the peak at 1637 cm^{-1} indicating the double bond in the active compound. C-H and C=C stretching in aromatic ring was showed by peaks at 3076, 1612, 1451, 1464 and 1514 cm^{-1}. Presence of methoxy group was indicated by C-O-C stretching at 1034cm^{-1}. The spectral data of the isolated compound is in agreement with that of eugenol reported in literature thus confirming the compound to be eugenol [11].

3.1.3 ^{1}H-NMR data

^{1}HNMR studies reveals that the presence of two protons at δ 6.68 may be present at third and fifth position whereas proton at δ 6.84 may be present at C-6 position indicating the presence of an aromatic ring in the molecule which is substituted at 3 positions [12].

Any factor responsible for shielding or deshielding of a proton will affect its chemical shift. One of the factors contributing for the degree of shielding is electron density around the proton. The higher the electron density around a proton, the higher the shielding and higher is the field (lower the δ value) at which the proton absorbs. Therefore the methoxy group on aromatic ring is represented by singlet of three protons at δ 3.87 and singlet proton at δ 5.490 indicates presence of aromatic alcoholic proton (Fig. 4). Corresponding to the doublet from δ 3.31 to δ 3.33 with coupling constant of 16.7 Hz indicates the presence of 2 protons on seventh carbon, suggesting $-CH_2$ group attached to aromatic ring. Chemical shift from δ 5.09 to δ 5.03 indicates the presence of 2 proton on ninth carbon andmultiplet at δ 6.0 to δ 5.89 on eighth carbon suggested the presence of exocyclic double bond. All these factors indicated the possible presence of allylicgroup. The deshielding effect on proton multiplet from δ 6.0 to δ 5.89 could be due to the influence of aromatic ring on it. Based upon these results and comparison with the values given in literature this compound was identified as eugenol [11,13].

3.1.4 ^{13}C-NMR data

The ^{13}C-NMR showed the presence of ten carbon environments [12]. Peaks at 39.9 ppm, 115.34 ppm, 137.8 ppm corresponding to -CH_2 carbon, =CH_2 carbon and =CH- carbon respectively further confirms the presence of allylic group (Fig. 5). Peaks at δ: 143.89, 146.43, 111.09, 131.93, 121.18, 114.24 indicated presence of six aromatic cabons, thus confirming the aromatic ring in the molecule. ^{13}C-NMR spectrum for eugenol molecule had similar characteristic peak as reported by Rahimi et al. [11].

3.1.5. DEPT data

DEPT showed the presence of one primary, two secondary, four tertiary C atoms. But spectra of ^{13}C-NMR indicates presence of 10 carbon environment (Fig. 6) .Thus from ^{13}C-NMR and DEPT spectra, presence of three quaternary carbons at δ: 143.89 (C-1), 146.43 (C-2) and 131.93 (C-4) that corresponds substitution at 1, 2 and 4 positions on benzene ring can be concluded .

The above spectral data is also supported by the data of ^{13}C-NMR and DEPT given in literature [11,12], hence confirming that the bioactive compound obtained by single spot isolation through Thin Layer Chromatography is eugenol.

3.1.6 Mass data

Rahimi et al. [11] reported characteristic peaks at m/z: 164 [$M^{\cdot+}$, 100%], 149 [$(M\text{-}CH_3)^+$], 137 [$(M\text{-}CH{=}CH_2)^+$], 121 [149-CO]$^+$, 131, 103 and 91. Fig.7showed the Mass spectrum of purified active compound. Molecular ion peak was observed at m/z 164 [$M^{\cdot+}$], that was also found to be the most abundant peak (100%). Other important peaks were observed at m/z: 149, 137, 121 due to removal of CH_3, C_2H_3 (from allylic group) and CO groups respectively. The peaks at 131, 103, 91 and 77 were also found in the range 30% - 40% abundance. Thus, from mass spectral data, the molecular weight of the pure active compound was tentatively confirmed same as Eugenol in literature.

3.1.7 Elemental data

The elemental analysis data is in conformity with the calculated percentages of carbon, hydrogen and oxygen present in eugenol (Table 1).In point of view of above discussion on the spectral and elemental analysis of the active compound isolated through column and thin layer chromatography, it is clearly established that the compound is Eugenol(4-allyl-2-methoxyphenol) [Fig. 8].

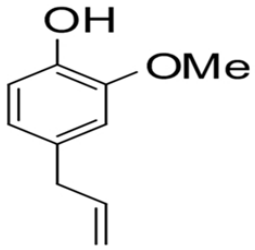

Fig. 8. Eugenol (4-allyl-2-methoxyphenol) isolated from *O. sanctum* essential oil

Table 2. Fungitoxic activity of eugenol isolated from *O. sanctum* leaves essential oil against phytopathogenic fungi of rice and rice based crop groundnut

Concentration (%)	Pathogen				
	P. grisea	***H. oryzae***	***R. solani***	***C. lunata***	***A. niger***
	Mycelial growth (cm/cm^2)				
1	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.2
0.1	1.6 (2.0)	2.5 (4.9)	2.0 (3.1)	2.5 (4.9)	3.8
0.01	3.8 (11.3)	4.3 (14.5)	4.5 (15.9)	4.2 (13.8)	11.9
0.001	4.5 (15.9)	4.5 (15.9)	4.5 (15.9)	4.5 (15.9)	15.9
Control	4.5 (15.9)	4.5 (15.9)	4.5 (15.9)	4.5 (15.9)	15.9

C.D. at P = 0.05 = 0.4. No mycelial growth was accorded the value 0.5 cm/ 0.2 cm^2. Data in parentheses represents area of mycelial growth in cm^2 calculated through 3.14×r^2 method [10] except in case of A. niger where area was calculated as discussed elsewhere in the text.

Phenolic compounds are important for the physiology of plants to contribute to resistance to microorganisms that can affect [14,15], help to preserve integrity of plants with continuous exposure to environmental stressors including radiation ultraviolet, relatively high temperatures and dehydration [16]. These are therefore active in biological systems having protective effect against different cardiovascular and neurological diseases and other dysfunctions based on oxidative stresses [17,18].

Antimicrobial activity was reported due to eugenol against*Saccharomyces cerevisiae,* and *Proteus mirabilis* exhibiting distortion in cell wall and membrane [19, 20]. Eugenol at 1% concentration exhibited complete mycelial growth inhibition (0.2cm^2±0.4) in all five phytopathogenic fungi (Table 2). Significant reduction of mycelial growth (11.3 cm^2±0.4 in *P. grisea*, 14.5 cm^2±0.4 in *H. oryzae*, 13.8 cm^2±0.4 in *C. lunata*, 11.9 cm^2±0.4 in *A. niger*) was exhibited at 0.01% concentration in all the test pathogens except *R. solani* that exhibited mycelial growth at par with control (15.9 cm^2±0.4). Thus revealing the fungitoxicity of eugenol against all the five phytopathogenic fungi.

4. CONCLUSION

Active compound was isolated and purified from essential oil of *O. sanctum* leaves through column chromatography and Thin Layer Chromatography. Through spectral techniques viz. FT-IR, UV, ^{1}H-NMR, ^{13}C-NMR, DEPT, Mass and elemental analysis, the compound was tentatively elucidated as eugenol. Further bioactivity of eugenol was tested against mycelial growth of five phytopathogenic fungi *P. grisea*, *H. oryzae*, *R. solani*, *C. lunata* and *A. niger* responsible for causing serious diseases i.e. Blast, Brown-spot, Sheath blight, Grain discoloration and Collar-rot in rice and groundnut crops respectively. The results indicated that the eugenol is the key component in *O. sanctum* essential oil responsible for its bioactivity against the above stated pathogens.

ACKNOWLEDGEMENTS

Authors thank Dr. T. Mohapatra, Director, Central Rice Research Institute, Cuttack, Dr. Anand Prakash, HOD, Crop Protection Division, CRRI, Dr A.V. Suriyarao, Former Principal Scientist for statistical analysis of the data and to Dr. Totan Adak, Senior Scientist, CRRI, for tentative structural elucidation. Senior author thanks to Mr. Arjuni Moharana, Techical Asst., Crop Protection Division for his support during the course of work. Spectral and Elemental

analysis done by SAIF, Panjab University, Chandigarh, Panjab and STIC, Cochin University of Science and Technology, Cochin, Kerala are also duly thankfully acknowledged.

REFERENCES

1. Mondal Shankar, MirdhaBijaya R, Mahapatra Sushil C. The Science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). Indian J. Physiol. Pharmacol. 2009;53(4):291-306.
2. Bunrathep Supawan, Palanuvej Chanida, Ruangrungsi Nijsiri. Chemical composition and antioxidative activities of essential oils from four *Ocimum* species endemic to Thailand. J. Health Res. 2007;21(3):201-206.
3. Vijayalakshmi K, Mishra S D, Prasad S K. Nematicidal properties of some indigenous plant materials against IInd stage juveniles of *Meloidogyne incognita* (Kofoid and White) Chitwood. Ind J. Ento. 1979;41:326-331.
4. Rajapakse RHS, Ratnasekera D. Pesticidal potential of some selected tropical plant extracts against *Callosobruchus Maculates* (F) and *Callosobruchus chinensis* (L) (Coleoptera : Bruchidae). Tropical Agricultural Research & Extension. 2008;11:69-71
5. Kalyan Kumar P, Rupesh Kumar M, Kavitha K, Singh Jagdeesh, Khan Rawoof Pharmacological Actions of *Ocimum sanctum* - Review Article. International Journal Of Advances In Pharmacy, Biology And Chemistry. 2012;1(3):406-414.
6. Tewari SN. *Ocimum sanctum* L, a botanical fungicide for rice blast control. Tropical Science. 1995;35:263-273.
7. Tewari S N. Strategic Approach for Management of Rice Blast through Eco-friendly Botanical Products. CRRI Research Bulletin 1, Central Rice Research Institute, Indian Council of Agricultural Research, Cuttack, India. 2008.
8. Upadhyaya S, Jashoda B, Tewari S N. Integrated management of foliar blast through ecofriendly formulated product, Oscext-e developed from *Ocimum sanctum* ethanolic extract. Archives of Phytopathology And Plant Protection. 2012;45(19):2290-2300.
9. Nene YL, Thapliyal PL. Disease Fungicides in Plant Control, Oxford and IBH Publishing Co., New Delhi, Mumbai, Kolkata; 1979.
10. Tewari SN, Shukla HS. Improved method for bioassay of pesticides International Rice Research Newsletter. 1990;15(1):26-27.
11. Rahimi AA, Ashnagar A, Nikoei H. Isolation and characterization of 4-allyl-2-methoxyphenol (eugenol) from clove buds marketed in Tehran city of Iran. International Journal Of ChemTech Research. 2012;4(1):105-108.
12. Awasthi PK, Dixit SC, Dixit N, Sinha AK. Eugenol Derivatives as Future Potential Drugs. Journal Of Pharmacy Research. 2008;1(2):215-220.
13. Berger S, Sicker D. Classics in spectroscopy, Isolation and Structure Elucidation of Natural Products. WILEY-VCH, Weinheim, Germany; 2009.
14. Rodrigues Ana Sofia, Pérez-Gregorio María Rosa, García- Falcón Mercedes Sonia, Simal- GándaraJesús, Almeida Domingos P.F. Effect of post-harvest practices on flavonoid content of red and white onion cultivars. Food Control. 2010;21:878-884.
15. Haslem E. Natural polyphenols(vegetables tannins) as drugs: possible modes of action. Journal Of Natural Products. 1996;59(2):205-215.
16. Castillo F, Hernández D, Gallegos G, Rodríguez R, Aguilar CN. Antifungal properties of bioactive compounds from plants. In: Dhanasekaran D, Thajuddin N, Paneerselvam A editors. Fungicides for plants and animal diseases. In-Tech. 2012.
17. Pérez-Gregorio María Rosa, García- Falcón Mercedes Sonia, Simal- GándaraJesús, Rodrigues Ana Sofia, Almeida Domingos P.F. Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. Journal of Food Composition And Analysis. 2010;23:592-598.

18. Rodrigues Ana Sofia, Pérez-Gregorio María Rosa, García- Falcón Mercedes Sonia, Simal-GándaraJesús, Almeida Domingos P.F. Effect of meteorological conditions on antioxidants flavonoids in Portuguese cultivars of white and red onions. Food Chemistry. 2011;124:303-308.
19. Bennis S, Chami F, Chami N, Bouchikhi T, Remmal A. Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. Letters in Applied Microbiology. 2004;38:454–458.
20. Devi K, Sakthivel R, Nisha S, Suganthy N, Pandian S. Eugenol alters the integrity of cell membrane and acts against the nosocomial pathogen *Proteus mirabilis*. Arch Pharm Res. 2013;36(3):282-292.

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