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Research Article

Emerging Anti-Microbial Perspectives of Pyrrolidine Containing Murrayanine-Chalcone

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Abstract: The current research aimed at rational development of pyrrolidine containing murrayanine based chalcone compound (*E*)-1-(1-methoxy-9*H*-carbazol-3-yl)-3-(4-(pyrrolidin-1-yl)phenyl)prop-2-en-1-one through Claisen-Schmidt reaction where natural product murrayanine in ring-A and pyrrolidine containing acetophenone in the B-ring were fused and screened against various microbial species; *Staphylococcus aureus* (*S. aureus*, MTCC 3160), *Escherichia coli* (*E. coli*, MTCC 2961), *Aspergillus niger* (*A. niger*, MTCC 277), and *Candida albicans* (*C. albicans*, MTCC 227). The novel pyrrolidine containing chalcone expressed noteworthy anti-bacterial and anti-fungal effect against the screened microbes, although did not perform better than that of the marketed products (positive controls) in both potential and potency. The compound exhibited the highest activity against *E. coli* followed by *C. albicans* and *S. aureus*. The lowest activity was found for *A. niger*. The benzylideneacetophenone scaffold containing murrayanine in ring-A and pyrrolidine (heterocycle) in ring-B opened new perspectives of anti-microbial research by motivating and providing new research opportunities to the global researchers.

Keywords: *Murraya koenigii*, murrayanine, chalcone, pyrrolidine, antifungal, antibacterial.

INTRODUCTION

Murraya koenigii L. or curry tree (Family: Rutaceae) contains more than 20 types of alkaloid of carbazole scaffold having wide varieties of ethnopharmacological properties such as febrifuge, purgative, carminative, astringent, stomachic, and anthelmintic (Mahapatra *et al*., 2018). Murrayanine is the most popular carbazole-based alkaloid with anti-inflammatory, anti-infective, anti-diabetic, anti-cancer, anti-oxidant, etc (Shivhare *et al*., 2016). However, the reported pharmacological activities were not so pronounced and there is an immense need to rationally enhance the therapeutic activities by various strategies. Moving towards the road to augment the activity, numerous semi-synthetic derivatives of murrayanine have been developed by our research groups and screened for a number of imperative activities such as anti-microbial, anti-inflammatory, anti-convulsant, anti-diabetic, anti-proliferative, and anxiolytic, which displayed higher and better pharmacotherapeutic results than the parent compound (Mahapatra *et al*., 2017; Mahapatra *et al*., 2018a; Mahapatra *et al*., 2018b).

Chalcone or benzylideneacetophenone or prop-2-en-1-one is a well-known natural based scaffold in modern medicinal chemistry having multifarious therapeutic potentials like anti-inflammatory, anti-leishmanial, anti-cancer, anti-obesity, anti-malarial, anti-diabetic, anti-retroviral, analgesic, anti-trypanosomal, anti-oxidant, anti-tubercular, anti-gout, anti-arrhythmic, anti-platelet, anti-filarial, anti-parasite, anti-hypertensive, anti-angiogenic, anti-fungal, anti-hyperlipidemic, anti-bacterial, etc (Mahapatra *et al*., 2015; Mahapatra *et al*., 2015a; Mahapatra *et al*., 2015b; Mahapatra and Bharti, 2016; Mahapatra *et al*., 2017a).

The current research aimed at rational development of pyrrolidine containing murrayanine based chalcone compound through Claisen-Schmidt reaction where natural product murrayanine in ring-A and pyrrolidine containing acetophenone in the B-ring were fused and screened against various microbial species; *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Candida albicans*.

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MATERIALS AND METHODS

Chemicals and Instrumentation

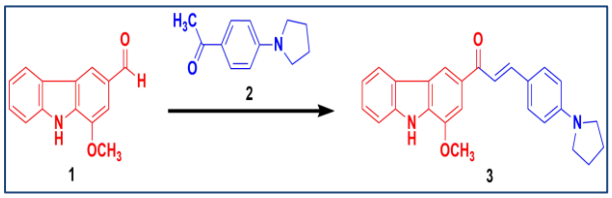
4′-(1-pyrrolidinyl)acetophenone, the reactant was purchased from Sigma Aldrich, Germany through a local vendor. HiMedia Ltd., India remained the chief vendor for the procurement of analytical grade chemicals, solvents, and reagents. The progress of the chemical reaction was determined by Merck® pre-coated Silica gel-G TLC plates. The elemental (CHN) analysis was performed by PerkinElmer 2400 model Elemental Analyzer. The compound was ascertained by spectroscopic analysis; Fourier transformed infrared spectroscopy (Shimadzu® IR-Affinity-1), ^{1}H-NMR spectroscopy (Bruker® Avance-II), and mass spectroscopy (MICROMASS Q-TOF).

Extraction of Murrayanine

Murrayanine. The starting material was extracted from the powdered stem bark of *M. koenigii* according to the method developed previously by our group (Mahapatra *et al*., 2017b). The phytoconstituent was isolated from the hexane fractions (B_{21}-B_{37}) by employing silica gel-based column chromatography. The content was further concentrated by the vacuum rotary evaporator.

Synthesis of Target Compounds

The chalcone scaffold **(3)** was fabricated by reacting the murrayanine **(1)**, the starting material with pyrrolidine containing acetophenone **(2)**, the reactant. The -$COCH_3$ (acetyl) part of the reactant reacts with the –CHO (aldehyde) portion of the starting material aldol condensation mechanism to form β-hydroxyketone function in the presence of ethanolic NaOH solution (**Scheme-1**).



Scheme-1. Development of pyrrolidine-murrayanine-chalcone.

Synthetic protocol for (*E*)-1-(1-Methoxy-9*H*-Carbazol-3-Yl)-3-(4-(Pyrrolidin-1-Yl) Phenyl) Prop-2-En-1-One (3)

Equal quantity of the starting material murrayanine **(1)** (0.01 M) and the reactant 4′-(1-pyrrolidinyl)acetophenone (0.01 M) **(2)** were refluxed in the presence of aqueous NaOH solution (20 mL) containing 90% ethanol (25 mL). The reaction content was made to stand for the whole night and further poured over crushed ice (containing a few drops of dilute HCl). The content was vigorously stirred using the glass rod to obtain the chalcone compound **(3)**. The product was separated through filtration, washed thoroughly to remove impurities, and suitably recrystallized (Mahapatra *et al*., 2018c).

75% yield; FTIR (KBr) υ (cm^{-1}): 3243 (-NH, stretching), 3051 (C-H, aromatic), 1714 (C=O), 1682 (C=C, alkene), 1611 (C=C, aromatic), 1590 (-NH, bending), 1344 (C-N), 1171 (C-O); ^{1}H-NMR (δ, ppm, $CDCl_3$): 10.16 (9, 1H), 6.7-8.1 (Aromatic, 10H), 3.85 (1, 3H), 3.59 (18, 2H), 1.99 (19, 2H). MS: M^+ 396. Anal. Calcd. for $C_{26}H_{24}N_2O_2$: C, 78.76; H, 6.10; N, 7.07. Found: C, 78.01; H, 5.89; N, 6.85.

Anti-Microbial Screening

The fabricated chalcone compound was screened by disc diffusion method against bacterial species *Staphylococcus aureus* (*S. aureus*, MTCC 3160) and *Escherichia coli* (*E. coli*, MTCC 2961) by employing the Muller Hinton Agar medium (incubation at 37±1ºC for 24 hrs) and also against fungal species *Aspergillus niger* (*A. niger*, MTCC 277) and *Candida albicans* (*C. albicans*, MTCC 227) by employing Potato Dextrose Agar medium (incubation at 37±1ºC for 72 hrs). The microbial species were initially cultured using the nutrient broth media at 37±1°C for 24 hr and then under laminar air flow condition, the content was transferred specifically into the agar plates. The compound was completely dissolved in dimethyl sulfoxide (DMSO), soaked over Whatman filter paper, placed carefully over the microbial plates and finally incubated (Kamble *et al*., 2017).

MIC (minimum inhibitory concentration) value was determined through agar streak dilution method. A microbial suspension of 10^5 CFU/mL concentration was prepared followed by serial dilution with DMSO. At 40-50ºC temperature, the suspension containing the test sample was suitably transferred into the petri dish at 5 mm depth. Ciprofloxacin (positive control for anti-bacterial studies), fluconazole (positive control for anti-fungal studies), and DMSO (negative control) were employed for screening. The average value of MIC was computed (Telrandhe *et al*., 2017).

RESULTS AND DISCUSSION

Chemistry

The structure of the chalcone compound was elucidated by the applications of spectroscopy. The disappearance of the aldehydic carbonyl group at 1753 cm^{-1} from the FT-IR spectra and appearance of a new ketonic carbonyl group at 1714 cm^{-1} represented the formation of the prop-2-ene-1-one scaffold. The carbazole portion was ascertained by the presence of methoxy component which appeared at 1171 cm^{-1} in the FT-IR and 3.85 ppm in the proton-NMR spectra. In addition to it, the –NH portion was substantiated by stretching and bending at 3243 cm^{-1} and 1590 cm^{-1} in the infrared spectra and 10.16 ppm in the ^{1}H-NMR spectra. The heterocycle-containing aromatic component was authenticated by the aromatic stretching in the range of 6.7 to 8.1 ppm. The aromatic portion

was additionally confirmed by the aromatic C-H and C=C stretching at 3051 cm^{-1} and 1611 cm^{-1} peaks. The protons of the five-membered component were corroborated at 3.59 ppm (position-18) and 1.99 ppm (position-19), respectively. The fabrication of the molecule was furthermore verified by the mass spectra which showed the emergence of the base peak corresponding to the base peak of the molecule, in addition to the fragmented products (m/z < 100). The ratio of CHN analysis provided a complete surety of the formation of the proposed compound.

Anti-Microbial Study

The novel pyrrolidine containing chalcone expressed noteworthy anti-bacterial and anti-fungal effect against the screened microbes, although did not perform better than that of the marketed products (positive controls) in both potential and potency. The compound exhibited the highest activity against *E. coli* followed by *C. albicans* and *S. aureus*. The lowest activity was found for *A. niger* (Table-1). The heterocycle-containing natural products based molecule has been found to articulate impressive anti-infective activity with low inter- and intra-variability. In the previous research done so far, the substitution of the heterocyclic six-membered piperidine and piperazine produced tremendous activity against the microbial species *E. coli*, *S. aureus*, *C. albicans*, and *A. niger* (Mahapatra *et al*., 2018d; Mahapatra *et al*., 2018e). The highest activity was displayed with the substitution of piperazine component followed by the piperidine component. However, replacement of the six-membered heterocycle by the five-membered heterocycle considerably reduces the bactericidal and fungicidal effect. It may be predicted that with an increase in the nitrogen atom, the activity increases. A structure-activity-relationship (SAR) can be predicted where the replacement to five membered component leads to a reduction in anti-microbial activity and increasing the nitrogen element in the ring enhances the activity significantly.

Table -1. Anti-microbial activities of pyrrolidine containing murrayanine-chalcone.

Compounds	***E. coli***	***S. aureus***	***A. niger***	***C. albicans***
3	23.97±1.33 (25)	18.76±1.36 (25)	17.44±1.61 (25)	20.96±1.66 (25)
Ciprofloxacin	32.82±1.51 (6.25)	31.27±1.79 (6.25)	-	-
Fluconazole	-	-	33.61±1.39 (6.25)	31.98±1.55 (6.25)

Zone of inhibition in millimeter, SD = standard deviation.

CONCLUSION

The fabricated pyrrolidine containing murrayanine-chalcone (*E*)-1-(1-methoxy-9*H*-carbazol-3-yl)-3-(4-(pyrrolidin-1-yl)phenyl)prop-2-en-1-one demonstrated noteworthy anti-bacterial and anti-fungal activity against *E. coli*, *S. aureus*, *C. albicans*, and *A. niger*, although the biological activity and therapeutic potency was found to be lesser than the positive controls (ciprofloxacin and fluconazole). The benzylideneacetophenone scaffold containing murrayanine in ring-A and pyrrolidine (heterocycle) in ring-B opened new perspectives of anti-microbial research by motivating and providing new research opportunities to the global researchers.

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CONFLICT OF INTEREST

No conflict of interest declared.

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